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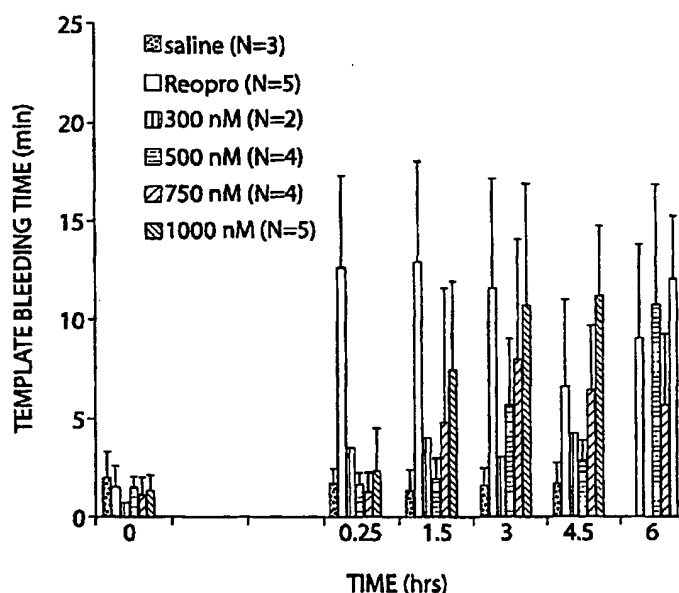
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(54) Title: APTAMERS TO VON WILLEBRAND FACTOR AND THEIR USE AS THROMBOTIC DISEASE THERAPEUTICS



(57) Abstract: The invention relates generally to the field of nucleic acids and more particularly to aptamers capable of binding to von Willebrand Factor useful as therapeutics in and diagnostics of thrombotic diseases and/or other diseases or disorders in which von Willebrand Factor mediated platelet aggregation has been implicated. The invention further relates to materials and methods for the administration of aptamers capable of binding to von Willebrand Factor.

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Aptamers to von Willebrand Factor And their Use As Thrombotic Disease Therapeutics

FIELD OF THE INVENTION

[0001] The invention relates generally to the field of nucleic acids and more particularly to aptamers capable of binding to von Willebrand Factor useful as therapeutics in and diagnostics of thrombotic diseases and/or other diseases or disorders in which von Willebrand Factor mediated platelet aggregation is implicated. The invention further relates to materials and methods for the administration of aptamers capable of binding to von Willebrand Factor.

BACKGROUND OF THE INVENTION

[0002] Aptamers are nucleic acid molecules having specific binding affinity to molecules through interactions other than classic Watson-Crick base pairing.

[0003] Aptamers, like peptides generated by phage display or monoclonal antibodies ("mAbs"), are capable of specifically binding to selected targets and modulating the target's activity or binding interactions, *e.g.*, through binding aptamers may block their target's ability to function. Discovered by an *in vitro* selection process from pools of random sequence oligonucleotides, aptamers have been generated for over 130 proteins including growth factors, transcription factors, enzymes, immunoglobulins, and receptors. A typical aptamer is 10-15 kDa in size (20-45 nucleotides), binds its target with nanomolar to sub-nanomolar affinity, and discriminates against closely related targets (*e.g.*, aptamers will typically not bind other proteins from the same gene family). A series of structural studies have shown that aptamers are capable of using the same types of binding interactions (*e.g.*, hydrogen bonding, electrostatic complementarities, hydrophobic contacts, steric exclusion) that drive affinity and specificity in antibody-antigen complexes.

[0004] Aptamers have a number of desirable characteristics for use as therapeutics and diagnostics including high specificity and affinity, biological efficacy, and excellent

pharmacokinetic properties. In addition, they offer specific competitive advantages over antibodies and other protein biologics, for example:

[0005] 1) Speed and control. Aptamers are produced by an entirely *in vitro* process, allowing for the rapid generation of initial leads, including therapeutic leads. *In vitro* selection allows the specificity and affinity of the aptamer to be tightly controlled and allows the generation of leads, including leads against both toxic and non-immunogenic targets.

[0006] 2) Toxicity and Immunogenicity. Aptamers as a class have demonstrated therapeutically acceptable toxicity and lack of immunogenicity. Whereas the efficacy of many monoclonal antibodies can be severely limited by immune response to antibodies themselves, it is extremely difficult to elicit antibodies to aptamers most likely because aptamers cannot be presented by T-cells via the MHC and the immune response is generally trained not to recognize nucleic acid fragments.

[0007] 3) Administration. Whereas most currently approved antibody therapeutics are administered by intravenous infusion (typically over 2-4 hours), aptamers can be administered by subcutaneous injection (aptamer bioavailability via subcutaneous administration is >80% in monkey studies (Tucker *et al.*, J. Chromatography B. 732: 203-212, 1999)). With good solubility (>150 mg/mL) and comparatively low molecular weight (aptamer: 10-50 kDa; antibody: 150 kDa), a weekly dose of aptamer may be delivered by injection in a volume of less than 0.5 mL. In addition, the small size of aptamers allows them to penetrate into areas of conformational constrictions that do not allow for antibodies or antibody fragments to penetrate, presenting yet another advantage of aptamer-based therapeutics or prophylaxis.

[0008] 4) Scalability and cost. Therapeutic aptamers are chemically synthesized and consequently can be readily scaled as needed to meet production demand. Whereas difficulties in scaling production are currently limiting the availability of some biologics and the capital cost of a large-scale protein production plant is enormous, a single large-scale oligonucleotide synthesizer can produce upwards of 100 kg/year and requires a relatively modest initial investment. The current cost of goods for aptamer synthesis at the kilogram scale is estimated at \$500/g, comparable to that for highly optimized antibodies. Continuing improvements in process development are expected to lower the cost of goods to < \$100/g in five years.

[0009] 5) Stability. Therapeutic aptamers are chemically robust. They are intrinsically adapted to regain activity following exposure to factors such as heat and denaturants and can be stored for extended periods (>1 yr) at room temperature as lyophilized powders.

THROMBOTIC DISEASE

[0010] During normal, controlled hemostasis, platelets do not adhere to healthy vessels, rather platelets typically adhere to the subendothelium of injured vessels. Platelet adhesion triggers a series of platelet activation processes which ultimately results in thrombus formation and cessation of bleeding. The von Willebrand Factor ("vWF") is a mediator of platelet adhesion at sites of vascular damage. vWF is a large multi-subunit, multimeric soluble factor mainly produced by vascular endothelial cells. The von Willebrand Factor becomes immobilized on the blood vessel wall via interactions between von Willebrand Factor domain A3 and exposed collagen. Transient interactions of the platelet-receptor glycoprotein Ib ("hereinafter GPIb") and the A1 domain of the immobilized von Willebrand Factor facilitates the adhesion and activation of platelets at sites of vascular injury. E.G. Huizinga *et al.*, Science, 297, 1176 (2002). Accordingly, the von Willebrand factor is pro-thrombotic, playing an important role during hemostasis in facilitating thrombus formation at sites of vascular injury.

[0011] Conversely, the von Willebrand Factor, by the same mechanism, also plays a key role in pathological conditions, such as cardiovascular diseases, involving platelet aggregation and thrombosis formation. Although antithrombotic therapies are currently available there is still a large unmet need for additional therapies. The American Heart Association estimates that more than 60 million people in the United States alone have one or more forms of cardiovascular disease, and that a high proportion of people with cardiovascular disease are at higher risk for arterial thrombosis. S.P. Jackson and S.M. Schoenwaelder, Nature Reviews, 2, 1-12 (2003).

[0012] A significant problem with presently available therapies is that improving efficacy reduces safety. S.P. Jackson and S.M. Schoenwaelder, Nature Reviews, 2, 1-12 (2003). Accordingly, it would be beneficial to treat or prevent thrombotic disease by preventing platelet aggregation in the vasculature while minimizing bleeding side effects. The present invention provides materials and methods to meet these and other needs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 is a schematic representation of the *in vitro* aptamer selection (SELEX™) process from pools of random sequence oligonucleotides.

[0014] Figure 2 is an illustration depicting various PEGylation strategies representing standard mono-PEGylation, multiple PEGylation, and oligomerization via PEGylation.

[0015] Figure 3 is a table listing the amino acid sequences of the von Willebrand Factor domain A1 proteins used in the experiments of the invention.

[0016] Figure 4 is a table listing the amino acid sequence of the full length human von Willebrand Factor protein used in the experiments of the invention.

[0017] Figure 5 is an illustration depicting the proposed secondary structure of ARC1029 (SEQ ID NO 214).

[0018] Figure 6 is a graph of the dot blot binding curves for ARC1029 (SEQ ID NO 214) to full length vWF and rabbit vWF domain A1. A black box in this table indicates a deletion.

[0019] Figure 7 is a graph of FACS data showing that ARC1029 (SEQ ID NO 214) inhibits binding of human vWF and rabbit vWF A1 domain to lyophilized human platelets.

[0020] Figure 8 is a graph of an aggregometer trace showing ARC1029 (SEQ ID NO 214) inhibiting botrocetin induced platelet aggregation over time.

[0021] Figure 9 is a graphical representation of ARC1029 (SEQ ID NO 214) inhibiting botrocetin induced platelet aggregation.

[0022] Figure 10 is an illustration depicting the proposed secondary structure for the SEQ ID NO 217 wherein C= fC, T= fU, N= any nucleotide but in paired regions it assumes a Watson/Crick base pair, and X= 1 to 4 nucleotides or Nx-Nx-Nx-Nx can be replaced with a PEG spacer.

[0023] Figure 11 is an illustration depicting the sequence alignment for three aptamers of the vWF rRdY SELEX™ Family #1.

[0024] Figure 12 is an illustration depicting the proposed secondary structure for SEQ ID NO 218.

[0025] Figure 13 is an illustration depicting the proposed secondary structure for SEQ ID NO 219.

[0026] Figure 14 is an illustration depicting the sequence alignment for 26 aptamers of the vWF DNA SELEX™ 2 Family #1. The black line at the top of the alignment represents the proposed core nucleic acid binding sequence required to bind the von Willebrand Factor target.

[0027] Figure 15 is an illustration depicting the proposed secondary structure for SEQ ID NO 220. Figure 15B is an illustration depicting the secondary structure of ARC1172 (SEQ ID NO 222) and which residues are tolerant of 2'-OMe substitution.

[0028] Figure 16 is a table depicting the nucleic acid sequences including any modifications of ARC1029 (SEQ ID NO 214), ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), and ARC1194 (SEQ ID NO 223) to ARC1243 (SEQ ID NO 272).

[0029] Figure 17 is a table depicting the nucleic acid sequences including any modifications of ARC1172 (SEQ ID NO 222), ARC1338 (SEQ ID NO 273) to ARC1348 (SEQ ID NO 283) and ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304). A black block in this table indicates a deletion. An "s" preceding a nucleotide indicator (e.g. T or dA) indicates a phosphorothioate substitution in the phosphate backbone 5' to the indicated nucleotide.

[0030] Figure 18 is a table depicting the nucleic acid sequences including any modifications of ARC1172 (SEQ ID NO 222), ARC1524 (SEQ ID NO 305) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317) and ARC1759 (SEQ ID NO 318). A black block in this table indicates a deletion.

[0031] Figure 19 is an illustration of the secondary structures of ARC1368 (SEQ ID NO 291) and ARC1534 (SEQ ID NO 315).

[0032] Figure 20 is a graph depicting the clotting time, in the PFA-100 assay, for human whole blood treated with ARC1368 (SEQ ID NO 291) or ARC1525 (SEQ ID NO 306) as a function of aptamer concentration.

[0033] Figure 21 is a graph depicting occlusion time, in a PFA-100 assay, of human whole blood treated with Integrilin™, ReoPro™ or ARC1368 (SEQ ID NO 291), as a function of drug concentration.

[0034] Figure 22 is a graph depicting percent inhibition, in BIPA, of human PRP, treated with Integrilin™, ReoPro™ or ARC1368 (SEQ ID NO 291), as a function of drug concentration.

[0035] Figure 23 is a graph depicting percent inhibition, in AIPA, of human PRP, treated with Integrilin™, ReoPro™ or ARC1368 (SEQ ID NO 291), as a function of drug concentration.

[0036] Figure 24 is a graph depicting percentage of full length ARC1172 (SEQ ID NO 222) or ARC1368 (SEQ ID NO 291), detected in human plasma, as a function of time.

[0037] Figure 25 is a graph depicting primate plasma aptamer concentration (determined using Oligreen analysis) plotted as a function of time following administration of ARC1368 (SEQ ID NO 291), ARC1779 (SEQ ID NO 320) or ARC1780 (SEQ ID NO 321).

[0038] Figure 26 is a graph showing the time points on the horizontal axis at which blood for testing was drawn from three cynomolgus macaques, ARC1779 (SEQ ID NO 320) plasma concentration (in nM) along the top third of the vertical axis, PFA-100 closure time (in seconds) on the middle third of the vertical axis, and the template or cutaneous bleeding time (in minutes) on the bottom third of the vertical axis. The average from all three animals for plasma aptamer concentration, PFA-100 closure time and cutaneous bleeding time is plotted on the top third, middle third and bottom third of the graph, respectively.

[0039] Figure 27 is a table showing the cutaneous bleeding time (CBT) in minutes, raw BIPA data and PFA-100 closure time (sec) at various time points, shown in column 1, relative to ARC1779 (SEQ ID NO 320) dosing in three different cynomolgus macaques.

[0040] Figure 28 is a graph showing the average PFA-100 closure time at various time points following ARC1779 (SEQ ID NO 320) dosing of C. macaques.

[0041] Figure 29 is a graph showing the average bleeding time of the three ARC1779 (SEQ ID NO 320) treated macaques taken at various time points following dosing.

[0042] Figure 30 is a graph correlating the average bleeding time in ARC1779 (SEQ ID NO 320) treated *C. macaques* (left vertical axis) to the PFA-100 closure time.

[0043] Figure 31 is a schematic depicting the blood sample collection schedule used in the assessment of ARC1779 (SEQ ID NO 320) in the cynomolgus monkey electrolytic thrombosis model.

[0044] Figure 32 is graph of ARC1779 (SEQ ID NO 320) plasma concentration (vertical axis) as a function of time in each cynomolgus monkey of treatment group 3 tested in the electrolytic thrombosis model.

[0045] Figure 33 is a graph of the time to occlusion of the right (hatched bar) or left carotid artery (indicated by a solid bar) in each cynomolgus macaque from each treatment group tested in the cynomolgus monkey electrolytic thrombosis model. Bar pairs 1, 8 and 9 indicate treatment group 1 (vehicle only) Bar pairs 2, 10, 11, 12 and 13 indicates treatment groups 2 and 4 (ReoPro). Bar pairs 3 to 7 indicate treatment group 3 (1000nM aptamer plasma concentration target group). Bar pairs 20, 22, 16, and 18 indicate treatment group 7 (750 nM plasma aptamer concentration target group) . Bar pairs 19, 21, 23 and 24 indicate treatment group 6 (500 nM plasma aptamer concentration target group). Bar pairs 15 and 17 indicate treatment group 5 (300 nM plasma aptamer concentration target group)

[0046] Figure 34 is a graph showing the cutaneous bleed time in minutes (vertical axis) of the various cynomolgous treatment groups in the electrical injury model taken at the time points shown on the horizontal axis.

SUMMARY OF THE INVENTION

[0047] The present invention provides materials and methods for the treatment of thrombotic disorders involving von Willebrand Factor mediated platelet aggregation.

[0048] The present invention provides aptamers that specifically bind to a von Willebrand Factor target. In some embodiments, the von Willebrand Factor target is human von Willebrand Factor. In some embodiments, the von Willebrand Factor target is a variant of human von Willebrand Factor that performs a biological function that is essentially the same

as a function of human von Willebrand Factor. In some embodiments, the biological function of the von Willebrand Factor target or variant thereof is to mediate platelet aggregation. In some embodiments, the variant of the human von Willebrand Factor target has substantially the same structure and substantially the same ability to bind an aptamer of the invention as that of human von Willebrand Factor. In some embodiments, the vWF target is a non-human von Willebrand Factor. In some embodiments, the aptamer of the invention binds the von Willebrand Factor target or a variant thereof that comprises an amino acid sequence which is at least 75%, 80%, 90% or 95% identical to SEQ ID NO 7 (Figure 4). In one embodiment, the von Willebrand Factor target comprises the amino acid sequence of SEQ ID NO 7.

[0049] The terms "sequence identity" or "% identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally, Ausubel, F. M. *et al.*, *Current Protocols in Molecular Biology*, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987)).

[0050] One example of an algorithm that is suitable for determining percent sequence identity is the algorithm used in the basic local alignment search tool (hereinafter "BLAST"),

see, *e.g.* Altschul *et al.*, J Mol. Biol. 215: 403-410 (1990) and Altschul *et al.*, Nucleic Acids Res., 15: 3389-3402 (1997). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (hereinafter "NCBI"). The default parameters used in determining sequence identity using the software available from NCBI, *e.g.*, BLASTN (for nucleotide sequences) and BLASTP (for amino acid sequences) are described in McGinnis *et al.*, Nucleic Acids Res., 32: W20-W25 (2004). In a preferred embodiment, percent identity is determined using the BLAST implemented algorithm of Altschul *et al.*, and the default parameters of McGinnis *et al.*, which herein may be referred to as BLAST percent identity.

[0051] In one embodiment, the vWF aptamer of the invention comprises a dissociation constant for human von Willebrand Factor or a variant thereof, of about 100 nM or less, preferably 50 nM or less, preferably 10 nM or less, preferably 5 nM or less, preferably 1 nM or less, and more preferably 500 pM or less. The dissociation constant may be determined by dot blot assay using a multi-point titration and fitting the equation $y = \frac{\text{max}}{(1 + K/\text{protein})} + y_{\text{int}}$ as described in Example 1, below.

[0052] The present invention also provides an aptamer that specifically binds to a von Willebrand Factor domain A1 target. In some embodiments, the von Willebrand Factor domain A1 target is a human von Willebrand Factor domain A1 target. In some embodiments, the human von Willebrand Factor domain A1 target is a variant of human von Willebrand Factor domain A1 that performs a biological function that is essentially the same as a function of human von Willebrand Factor domain A1. In some embodiments, the biological function of von Willebrand Factor domain A1 or a variant thereof is to bind to platelets. In some embodiments, the variant of human von Willebrand Factor domain target has substantially the same structure and substantially the same ability to bind said aptamer as that of human von Willebrand Factor domain A1. In other embodiments, the von Willebrand Factor domain A1 target is non-human von Willebrand Factor domain A1 target, *e.g.* a rabbit or non-human primate von Willebrand Factor domain A1 target.

[0053] In some embodiments, the von Willebrand Factor domain A1 target of the invention comprises an amino acid sequence which is at least 75%, 80%, 90% or 95% identical to any one of the sequences selected from the group consisting of: SEQ ID NOS 4 to

6. In a preferred embodiment, the von Willebrand Factor domain A1 target comprises any one of the amino acid sequences selected from the group consisting of SEQ ID NOS 4 to 6.

[0054] In one embodiment, the vWF aptamer of the invention comprises a dissociation constant for human von Willebrand Factor domain A1 or a variant thereof, of about 100 nM or less, preferably 50 nM or less, preferably 10 nM or less, preferably 5 nM or less, preferably 1 nM or less, and more preferably 500 pM or less. In another embodiment, the aptamer of the invention comprises a dissociation constant for non-human von Willebrand Factor domain A1 or a variant thereof, of about 100 nM or less, preferably 50 nM or less, preferably 10 nM or less, preferably 5 nM or less, preferably 1 nM or less, and more preferably 500 pM or less. The dissociation constant may be determined by dot blot assay using a multi-point titration and fitting the equation $y = (\text{max}/(1+K/\text{protein})) + y_{\text{int}}$ as described in Example 1, below.

[0055] In some embodiments, the invention provides an aptamer that specifically binds to a von Willebrand Factor full length target. In some embodiments, the invention provides an aptamer that specifically binds to a von Willebrand Factor full length target and a von Willebrand Factor domain A1 target. In some embodiments, the von Willebrand Factor full length target is a human von Willebrand target or variant thereof. In other embodiments, the von Willebrand Factor full length target is a non-human von Willebrand target or variant thereof. In some embodiments, the von Willebrand Factor domain A1 target is a non-human von Willebrand Factor domain A1 target or variant thereof. In other embodiments, the von Willebrand Factor domain A1 target is a human von Willebrand Factor domain A1 target or variant thereof. In some embodiments, the von Willebrand Factor full length target or domain A1 target is selected from the group consisting of: a rabbit, guinea pig, monkey, dog, sheep, mouse and rat, von Willebrand Factor full length or domain A1 target. In some embodiments, the von Willebrand Factor full length target and von Willebrand Factor domain A1 target to which the aptamer of the invention specifically binds, are from different species.

[0056] The present invention provides aptamers against a von Willebrand Factor target that are ribonucleic acid or deoxyribonucleic acid or mixed ribonucleic acid and deoxyribonucleic acid. Aptamers of the invention may be single stranded ribonucleic acid or deoxyribonucleic acid or mixed ribonucleic acid and deoxyribonucleic acid. In some

embodiments, the aptamer of the invention comprises at least one chemical modification. In some embodiments, the chemical modification is selected from the group consisting of: a chemical substitution at a sugar position; a chemical substitution at a phosphate position; and a chemical substitution at a base position, of the nucleic acid. In other embodiments, the chemical modification is selected from the group consisting of: incorporation of a modified nucleotide, 3' capping, conjugation to a high molecular weight, non-immunogenic compound, conjugation to a lipophilic compound, and incorporation of phosphorothioate into the phosphate back bone. In a preferred embodiment, the non-immunogenic, high molecular weight compound is polyalkylene glycol, more preferably polyethylene glycol.

[0057] In some embodiments of the present invention, an aptamer, *e.g.* a von Willebrand Factor aptamer, that binds specifically to a target wherein the aptamer comprises a nucleotide sequence having no more than four, no more than three, no more than two or no more than one phosphorothioate backbone modifications, and the aptamer has a binding affinity for the target wherein the binding affinity is increased relative to a second aptamer having the same nucleotide sequence but lacking phosphorothioate back bone modification is provided. In some embodiments, the target is a protein or peptide not having the known function of binding nucleic acid, particularly not have the known primary function of binding nucleic acid.

[0058] In some embodiments, the aptamer of the invention modulates a function of any one of the group consisting of: the von Willebrand Factor target, the von Willebrand Factor domain A1 target and a variant of either target. In some embodiments, the modulated function is platelet aggregation mediation. In some embodiments, the aptamer of the invention inhibits von Willebrand Factor mediated platelet aggregation *in vivo*. In other embodiments, the aptamer of the invention prevents binding of any one of the group consisting of: the von Willebrand Factor target, the von Willebrand Factor domain A1 target and a variant thereof, to a platelet. In other embodiments, the aptamer of the invention prevents binding of any one of the group consisting of: the von Willebrand Factor target, the von Willebrand Factor domain A1 target and a variant of either target, to a platelet receptor protein. In yet other embodiments, the aptamer of the invention prevents binding of any one of the group consisting of: the von Willebrand Factor target, the von Willebrand Factor domain A1 target

and a variant of either target, to the platelet receptor protein GPIb. In some embodiments, the aptamer of the invention prevents vWF Factor mediated platelet aggregation while not significantly increasing bleeding time. In some embodiments, a non-significant increase in bleeding time is less than 15, minutes, preferably less than 10 minutes, more preferably less than 5 minutes, and in some embodiments, less than 3 minutes relative to the bleeding time of a subject not treated with the aptamer of the invention. In some embodiments the bleeding time is determined by cutaneous (or template) bleeding time.

[0059] In some embodiments, the aptamer of the invention has substantially the same ability to bind any one of the group consisting of: the von Willebrand Factor target, the von Willebrand Factor domain A1 target and a variant thereof, as that of an aptamer selected from the group consisting of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In other embodiments, the aptamer of the invention has substantially the same structure and substantially the same ability to bind of any one of the group consisting of: the von Willebrand Factor target, the von Willebrand Factor domain A1 target and a variant thereof, as that of an aptamer selected from the group of sequences consisting of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ

ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In yet other embodiments, the aptamer of the invention is selected from the group consisting of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323).

[0060] In a particular embodiment, the aptamer of the invention comprises the primary nucleic acid sequence of ARC1172 (SEQ ID NO 222) or ARC1115 (SEQ ID NO 221) or ARC1029 (SEQ ID NO 214) or SEQ ID NO 220 and does not comprise a 2'-O-Me substituted nucleotide at position 6 to 9, 20, 22, 24 to 27, 30 or 32 to 33. In another embodiment, the aptamer of the invention comprises the nucleic acid sequence of ARC1172 (SEQ ID NO 222) or ARC1115 (SEQ ID NO 221) or ARC1029 (SEQ ID NO 214) or SEQ ID NO 220 and comprises a 2'-O-Me substituted nucleotide at one or more positions, at 5 or more positions, at 10 or more positions, at 15 or more positions, or at 20 or more positions. In another embodiment, the aptamer of the invention comprises the nucleic acid sequence of ARC1172 (SEQ ID NO 222) or ARC1115 (SEQ ID NO 221) or ARC1029 (SEQ ID NO 214) or SEQ ID NO 220 and comprises a 2'-O-Me substituted nucleotide at all positions selected from the group consisting of: position 1 to 5, position 10 to 19, position 21, position 23, position 28 to 29, and position 34 to 41 wherein the position numbering starts at the 5' end of the nucleic acid sequence.

[0061] In a particular embodiment of the invention, an aptamer comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO 95 to 97 and SEQ ID NO 217 to

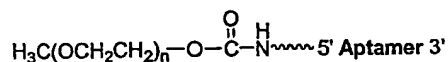
219 wherein: Y = C or T/U, R = A or G, N = any nucleotide but in paired regions it assumes a Watson/Crick base pair; and X = 1 to 4, is provided. In another embodiment, an aptamer of the invention is selected from the group consisting of: SEQ ID NO 217 and 220 wherein N_x-N_x-N_x-N_x, or N₍₃₋₁₀₎ may be replaced with a PEG linker. In yet another embodiment, an aptamer of the invention is selected from the group consisting of SEQ ID NOs 325-327, where Y = C or T, R = A or G.

[0062] In a particular embodiment, the aptamer that binds specifically to von Willebrand Factor comprises a three way helix junction secondary structure motif having the consensus sequence structure of SEQ ID NO 220 depicted in Figure 15. In another particular embodiment, the aptamer having the three way helix junction comprises the consensus structure depicted in Figure 19 A (ARC1368 (SEQ ID NO 291)). While in another embodiment, the aptamer having the three way helix junction comprises the consensus structure depicted in Figure 19 B (ARC1534 (SEQ ID NO 315)).

[0063] In another embodiment, the aptamer that binds specifically to von Willebrand Factor comprises a stem-loop-stem-loop secondary structure motif having the consensus sequence structure of SEQ ID NO 217 depicted in Figure 10. In another embodiment, the aptamer that specifically binds to von Willebrand Factor comprises the stem-loop-loop secondary structure motif having the consensus sequence structure of SEQ ID NO 218 depicted in Figure 12. In another embodiment, the aptamer that binds specifically to von Willebrand Factor comprises a three way junction secondary structure motif with two helical stems and a stem-loop of SEQ ID NO 19 as depicted in Figure 13. In some embodiments, the secondary structure motif of the aptamer of the invention is predicted by: RNAstructure, Version 4.1 (Mathews, D.H.; Disney, M.D.; Childs, J.L.; Schroeder, S.J.; Zuker, M.; and Turner, D.H., "Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure," 2004. *Proceedings of the National Academy of Sciences, US*, 101, 7287-7292).

[0064] In a preferred embodiment, an aptamer that specifically binds to a human von Willebrand Factor target and to a non-human von Willebrand Factor target is provided.

[0065] In one embodiment, an aptamer comprising the following structure or a salt thereof is provided:

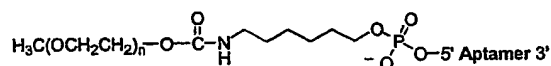


wherein: n is about 454 ethylene oxide units (PEG = 20 kDa)

$\sim\sim\sim$ is a linker,

and the aptamer is an anti-vWF aptamer of the invention. In a particular embodiment, the aptamer comprises the following nucleic acid sequence or fragment thereof: mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmG-s-dTmGdCdGdGTmGmCdCmUdCdCmGmUdCmAmCmGmC-3T (SEQ ID NO 291) wherein m refers to a 2'-OMe substitution, the "d" refers to a deoxy nucleotide, the "s" refers to a phosphorothioate substitution and "3T" refers to an inverted deoxy thymidine. In another embodiment, the aptamer comprises the following nucleic acid sequence or fragment thereof: dGdGdCdGdTdGdCdAdGdTdGdCdCdTdTdCdGdGdCdCdGdTdGdCdGdGdTdGdCdCdTdCdCdGdTdCdAdCdGdCdC-3T (SEQ ID NO 323) wherein "d" refers to a deoxy nucleotide and "3T" refers to an inverted deoxy thymidine. In some embodiments of this aspect of the invention the linker is an alkyl linker. In particular embodiments, the alkyl linker comprises 2 to 18 consecutive CH₂ groups. In preferred embodiments, the alkyl linker comprises 2 to 12 consecutive CH₂ groups. In particularly preferred embodiments the alkyl linker comprises 3 to 6 consecutive CH₂ groups.

[0066] In a particular embodiment, the aptamer of the invention comprises the following structure:



wherein: n is about 454 ethylene oxide units (PEG = 20 kDa),

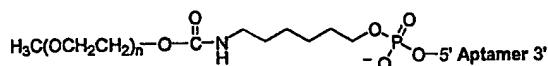
and the aptamer nucleic acid sequence is an anti-vWF aptamer of the invention. In a particular embodiment, the aptamer comprises the following nucleic acid sequence or fragment thereof: mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmG-s-dTmGdCdGdGTmGmCdCmUdCdCmGmUdCmAmCmGmC-3T (SEQ ID NO 291) wherein m refers to a 2'-OMe substitution, the "d" refers to a deoxy nucleotide, the "s" refers to a

phosphorothioate substitution and "3T" refers to an inverted deoxy thymidine. In another embodiment, the aptamer comprises the following nucleic acid sequence or fragment thereof: dGdGdCdGdTdGdCdAdGdTdGdCdCdTdTdCdGdGdCdCdGdTdGdCdGdGdTdGdCdCdTdc dCdGdTdCdAdCdGdCdC-3T (SEQ ID NO 323) wherein "d" refers to a deoxy nucleotide and "3T" refers to an inverted deoxy thymidine.

[0067] In another embodiment, a salt of an aptamer of the invention is provided. In a particular embodiment, the following salt of an aptamer is provided:

N-(methoxy-polyethyleneglycol)-6-aminohexyl-(1→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-uracylyl-(3'→5')-2'-deoxyguanylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-deoxyadenylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-uracylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-2'-OMe-uracylyl-(3'→5')-2'-OMe-uracylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-deoxythymidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-deoxyguanylyl-(3'→5')-2'-deoxythymidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-OMe-uracylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-uracylyl-(3'→5')-2'-deoxycytidylyl-(3'→5')-2'-OMe-adenylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-2'-OMe-guanylyl-(3'→5')-2'-OMe-cytidylyl-(3'→5')-(3'→3')-2'-deoxythymidine, 40-sodium salt, wherein the methoxy polyethyleneglycol comprises a molecular weight of 20 kDa.

[0068] In yet another embodiment, a pharmaceutical composition comprising a therapeutically effective amount of any one of the aptamers of the invention or a salt thereof and a pharmaceutically acceptable carrier or diluent is provided. In a particular embodiment, the pharmaceutical composition of the invention comprises ARC1779. In a more particular embodiment the pharmaceutical composition comprises an aptamer having the following structure or a salt thereof:



wherein: n is about 454 ethylene oxide units (PEG = 20 kDa), and the aptamer comprises the following nucleic acid sequence or fragment thereof:

mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmG-s-

dTmGdCdGdGTmGmCdCmUdCdCmGmUdCmAmCmGmC-3T (SEQ ID NO 291) wherein m refers to a 2'-OMe substitution, the "d" refers to a deoxy nucleotide, the "s" refers to a phosphorothioate substitution and "3T" refers to an inverted deoxy thymidine.

[0069] The invention provides a method of treating, preventing or ameliorating a disease mediated by von Willebrand Factor, comprising administering an aptamer or a pharmaceutical composition of the invention to a vertebrate, preferably a mammal, more preferably a human. In some embodiments, the disease to be treated, prevented or ameliorated is selected from the group consisting of: essential thrombocytopenia, thrombotic thrombocopenic purpura ("TTP"), Type IIb von Willebrand's disease, pseudo von Willebrand disease, peripheral artery disease, *e.g.* peripheral arterial occlusive disease, unstable angina, angina pectoris, arterial thrombosis, atherosclerosis, myocardial infarction, acute coronary syndrome, atrial fibrillation, carotid stenosis, cerebral infarction, cerebral thrombosis, ischemic stroke, and transient cerebral ischemic attack. In some embodiments, the pharmaceutical composition of the invention is administered prior to/during and/or after dialysis, CABG surgery, percutaneous coronary intervention or heart valve replacement.

[0070] The length of the *in vivo* half life of the aptamer of the invention may vary depending on the disease to be treated, ameliorated and/or prevented. For example, in some embodiments in which chronic aptamer administration is desirable due to the characteristics of the disease to be treated, ameliorated and/or prevented, the aptamer of the invention may comprise a relatively long half life, *e.g.* a half life greater than five hours in humans.

[0071] In other embodiments, the aptamer of the invention comprises a desired functional half life or duration of effect. Functional half life or duration of effect is a function of both pharmacokinetic half life and pharmacodynamic activity of the aptamer. In some embodiments, the desired human functional half-life or duration of effect for an anti-vWF therapeutic aptamer is on the order of 1-5 hours for the proposed indications elective PCI and ACS. Aptamers with such kinetics represent a balance between the dual objectives of (1) minimizing total aptamer dose (achieved with longer half-life) and (2) allowing rapid normalization of platelet function following cessation of treatment (achieved with shorter half-life). In some embodiments, rapid normalization of platelet function is important as it

allows clinicians the option of rapid intervention (*e.g.* CABG) should a patient fail to stabilize in response to treatment.

[0072] Accordingly, in some embodiments the aptamer for use in the methods of treatment and/or pharmaceutical compositions of the invention comprises a relatively short functional half life, *e.g.* a functional half life in humans of about 1 to 5 hours. In some embodiments, the functional half life in humans is at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours and not more than about 5 hours. In some embodiments, the functional half life or duration of effect is about the same as the distribution half life $T_{1/2\alpha}$ of the aptamer.

[0073] In some embodiments, the aptamer of the invention comprising the short functional half life in humans is for use in methods and compositions for the treatment, amelioration or prevention of diseases that potentially may require surgical intervention, such as acute coronary syndrome. In some embodiments, the aptamer of this aspect of the invention comprising a short functional half life in humans is conjugated to a PEG, *e.g.* a 5, 10 or 20 kDa PEG. In some embodiments, the aptamer of this aspect of the invention comprising a short half life in humans is ARC1779.

[0074] The invention also provides a diagnostic method comprising contacting an aptamer of the invention with a composition suspected of comprising von Willebrand Factor, von Willebrand Factor domain A1 or a variant thereof and detecting the presence or absence of von Willebrand Factor, von Willebrand Factor domain A1 or a variant thereof. In some embodiments, the diagnostic method is for use *in vitro* while in other embodiments, the diagnostic method is for use *in vivo*.

[0075] The invention also provides a method for identifying an aptamer that blocks a biological function *in vivo* comprising:

- a) preparing a candidate mixture of single-stranded nucleic acids;
- b) contacting the candidate mixture with both a full length protein target and a domain of the full length protein target;
- c) partitioning the nucleic acids having an increased affinity for the full length protein target or the protein target domain; and

d) amplifying the increased affinity nucleic acids, *in vitro*, to yield a protein target specific enriched aptamer mixture.

[0076] In some embodiments, the identification method further comprises;

e) contacting the target specific enriched aptamer mixture with the full length protein target;

f) partitioning the nucleic acids having an increased affinity for the full length protein target; and

g) amplifying the increased affinity nucleic acids, *in vitro*; to yield a target specific enriched aptamer mixture;

h) contacting the target specific enriched aptamer mixture with the protein target domain;

i) partitioning the nucleic acids having an increased affinity for the protein target domain; and

j) amplifying the increased affinity nucleic acids, *in vitro*, to yield a protein target specific enriched aptamer mixture.

[0077] In some embodiments, the identification method further comprises selecting an aptamer that blocks a biological function of the full length protein target *in vivo* while in other embodiments, the method further comprises selecting an aptamer that blocks a biological function of the protein target domain *in vivo*. In some embodiments of the identification method of the invention, the full length protein target is from a first species and the protein target domain is from a second species. In further embodiments of the identification method of the invention, the method further comprises selecting an aptamer capable of binding to both protein targets of both the first and second species, preferably selecting an aptamer that blocks a biological function of the protein target in both the first and second species. In some embodiments of the identification method of the invention, the full length target protein target is von Willebrand Factor. In some embodiments of the identification method of the invention, the full length target protein target is von Willebrand Factor, wherein it is preferred that the selected aptamer blocks von Willebrand Factor mediated platelet aggregation. In some

embodiments the protein target domain is von Willebrand Factor domain A1. The invention also provides an aptamer identified by the identification method of the invention.

[0078] In some embodiments, the invention also provides an aptamer that specifically binds to von Willebrand Factor comprising a primary nucleic acid sequence at least 80% identical, particularly at least 90% identical, and more particularly at least 95% identical to any one of the primary nucleic acid sequences selected from the group consisting of SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In some embodiments, the % sequence identity of the aptamers of the invention is BLAST sequence identity.

[0079] In another embodiment, the aptamer of the invention comprises a nucleic acid sequence having chemical modifications that including chemical modifications is at least 80% identical, particularly 90% identical, and more particularly at least 95% identical to any one of the nucleic acid sequences selected from the group consisting of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO

318), ARC1779 to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323).

[0080] In yet another embodiment, the invention provides an aptamer that upon binding a von Willebrand Factor target modulates a von Willebrand Factor function, preferably *in vivo* and comprises a sequence of 30 contiguous nucleotides that are identical to a sequence of 30 contiguous nucleotides comprised in any one of the sequences selected from the group of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In yet another embodiment, the aptamer of the invention upon binding a von Willebrand Factor target modulates a von Willebrand Factor function, preferably *in vivo*, and comprises 20 contiguous nucleotides that are identical to a sequence of 20 contiguous nucleotides in the unique sequence region of any one of the aptamer selected from the group of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In yet another embodiment, the aptamer of the invention upon binding a von Willebrand Factor target modulates a von Willebrand Factor function, preferably *in vivo*, and comprises 8

contiguous nucleotides that are identical to a sequence of 8 contiguous nucleotides in the unique sequence region of any one of the aptamer selected from the group of : SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In yet another embodiment, the aptamer of the invention upon binding a von Willebrand Factor target modulates a von Willebrand Factor function, preferably *in vivo*, and comprises 4 contiguous nucleotides that are identical to a sequence of 4 contiguous nucleotides in the unique sequence region of any one of the aptamer selected from the group of : SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323).

DETAILED DESCRIPTION OF THE INVENTION

[0081] The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the

preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present Specification will control.

THE SELEX™ METHOD

[0082] A suitable method for generating an aptamer is with the process entitled "Systematic Evolution of Ligands by Exponential Enrichment" ("SELEX™") generally depicted in Figure 1. The SELEX™ process is a method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules and is described in, *e.g.*, U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, now abandoned, U.S. Pat. No. 5,475,096 entitled "Nucleic Acid Ligands", and U.S. Pat. No. 5,270,163 (see also WO 91/19813) entitled "Nucleic Acid Ligands". Each SELEX™-identified nucleic acid ligand, *i.e.*, each aptamer, is a specific ligand of a given target compound or molecule. The SELEX™ process is based on the unique insight that nucleic acids have sufficient capacity for forming a variety of two- and three-dimensional structures and sufficient chemical versatility available within their monomers to act as ligands (*i.e.*, form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric. Molecules of any size or composition can serve as targets.

[0083] SELEX™ relies as a starting point upon a large library or pool of single stranded oligonucleotides comprising randomized sequences. The oligonucleotides can be modified or unmodified DNA, RNA, or DNA/RNA hybrids. In some examples, the pool comprises 100% random or partially random oligonucleotides. In other examples, the pool comprises random or partially random oligonucleotides containing at least one fixed sequence and/or conserved sequence incorporated within randomized sequence. In other examples, the pool comprises random or partially random oligonucleotides containing at least one fixed and/or conserved sequence at its 5' and/or 3' end which may comprise a sequence shared by all the molecules of the oligonucleotide pool. Fixed sequences are sequences common to oligonucleotides in the pool which are incorporated for a preselected purpose, such as CpG motifs described

further below, hybridization sites for PCR primers, promoter sequences for RNA polymerases (e.g., T3, T4, T7, and SP6), restriction sites, or homopolymeric sequences, such as poly A or poly T tracts, catalytic cores, sites for selective binding to affinity columns, and other sequences to facilitate cloning and/or sequencing of an oligonucleotide of interest. Conserved sequences are sequences, other than the previously described fixed sequences, shared by a number of aptamers that bind to the same target.

[0084] The oligonucleotides of the pool preferably include a randomized sequence portion as well as fixed sequences necessary for efficient amplification. Typically the oligonucleotides of the starting pool contain fixed 5' and 3' terminal sequences which flank an internal region of 30–50 random nucleotides. The randomized nucleotides can be produced in a number of ways including chemical synthesis and size selection from randomly cleaved cellular nucleic acids. Sequence variation in test nucleic acids can also be introduced or increased by mutagenesis before or during the selection/amplification iterations.

[0085] The random sequence portion of the oligonucleotide can be of any length and can comprise ribonucleotides and/or deoxyribonucleotides and can include modified or non-natural nucleotides or nucleotide analogs. See, e.g., U.S. Patent No. 5,958,691; U.S. Patent No. 5,660,985; U.S. Patent No. 5,958,691; U.S. Patent No. 5,698,687; U.S. Patent No. 5,817,635; U.S. Patent No. 5,672,695, and PCT Publication WO 92/07065. Random oligonucleotides can be synthesized from phosphodiester-linked nucleotides using solid phase oligonucleotide synthesis techniques well known in the art. See, e.g., Froehler *et al.*, Nucl. Acid Res. 14:5399-5467 (1986) and Froehler *et al.*, Tet. Lett. 27:5575-5578 (1986). Random oligonucleotides can also be synthesized using solution phase methods such as triester synthesis methods. See, e.g., Sood *et al.*, Nucl. Acid Res. 4:2557 (1977) and Hirose *et al.*, Tet. Lett., 28:2449 (1978). Typical syntheses carried out on automated DNA synthesis equipment yield 10^{14} - 10^{16} individual molecules, a number sufficient for most SELEX™ experiments. Sufficiently large regions of random sequence in the sequence design increases the likelihood that each synthesized molecule is likely to represent a unique sequence.

[0086] The starting library of oligonucleotides may be generated by automated chemical synthesis on a DNA synthesizer. To synthesize randomized sequences, mixtures of all four nucleotides are added at each nucleotide addition step during the synthesis process, allowing

for random incorporation of nucleotides. As stated above, in one embodiment, random oligonucleotides comprise entirely random sequences; however, in other embodiments, random oligonucleotides can comprise stretches of nonrandom or partially random sequences. Partially random sequences can be created by adding the four nucleotides in different molar ratios at each addition step.

[0087] The starting library of oligonucleotides may be either RNA or DNA, or substituted RNA or DNA. In those instances where an RNA library is to be used as the starting library it is typically generated by synthesizing a DNA library, optionally PCR amplifying, then transcribing the DNA library *in vitro* using T7 RNA polymerase or modified T7 RNA polymerases, and purifying the transcribed library. The RNA or DNA library is then mixed with the target under conditions favorable for binding and subjected to step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. More specifically, starting with a mixture containing the starting pool of nucleic acids, the SELEX™ method includes steps of: (a) contacting the mixture with the target under conditions favorable for binding; (b) partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; (c) dissociating the nucleic acid-target complexes; (d) amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids; and (e) reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule. In those instances where RNA aptamers are being selected, the SELEX™ method further comprises the steps of: (i) reverse transcribing the nucleic acids dissociated from the nucleic acid-target complexes before amplification in step (d); and (ii) transcribing the amplified nucleic acids from step (d) before restarting the process.

[0088] Within a nucleic acid mixture containing a large number of possible sequences and structures, there is a wide range of binding affinities for a given target. A nucleic acid mixture comprising, for example, a 20 nucleotide randomized segment can have 4^{20} candidate possibilities. Those which have the higher affinity (lower dissociation constants) for the target are most likely to bind to the target. After partitioning, dissociation and amplification,

a second nucleic acid mixture is generated, enriched for the higher binding affinity candidates. Additional rounds of selection progressively favor the best ligands until the resulting nucleic acid mixture is predominantly composed of only one or a few sequences. These can then be cloned, sequenced and individually tested for binding affinity as pure ligands or aptamers.

[0089] Cycles of selection and amplification are repeated until a desired goal is achieved. In the most general case, selection/amplification is continued until no significant improvement in binding strength is achieved on repetition of the cycle. The method is typically used to sample approximately 10^{14} different nucleic acid species but may be used to sample as many as about 10^{18} different nucleic acid species. Generally, nucleic acid aptamer molecules are selected in a 5 to 20 cycle procedure. In one embodiment, heterogeneity is introduced only in the initial selection stages and does not occur throughout the replicating process.

[0090] In one embodiment of SELEXTM, the selection process is so efficient at isolating those nucleic acid ligands that bind most strongly to the selected target, that only one cycle of selection and amplification is required. Such an efficient selection may occur, for example, in a chromatographic-type process wherein the ability of nucleic acids to associate with targets bound on a column operates in such a manner that the column is sufficiently able to allow separation and isolation of the highest affinity nucleic acid ligands.

[0091] In many cases, it is not necessarily desirable to perform the iterative steps of SELEXTM until a single nucleic acid ligand is identified. The target-specific nucleic acid ligand solution may include a family of nucleic acid structures or motifs that have a number of conserved sequences and a number of sequences which can be substituted or added without significantly affecting the affinity of the nucleic acid ligands to the target. By terminating the SELEXTM process prior to completion, it is possible to determine the sequence of a number of members of the nucleic acid ligand solution family.

[0092] A variety of nucleic acid primary, secondary and tertiary structures are known to exist. The structures or motifs that have been shown most commonly to be involved in non-Watson-Crick type interactions are referred to as hairpin loops, symmetric and asymmetric bulges, pseudoknots and myriad combinations of the same. Almost all known cases of such motifs suggest that they can be formed in a nucleic acid sequence of no more than 30

nucleotides. For this reason, it is often preferred that SELEX™ procedures with contiguous randomized segments be initiated with nucleic acid sequences containing a randomized segment of between about 20 to about 50 nucleotides, and of about 30 to about 40 nucleotides in some embodiments. In one example, the 5'-fixed:random:3'-fixed sequence comprises a random sequence of about 30 to about 50 nucleotides.

[0093] The core SELEX™ method has been modified to achieve a number of specific objectives. For example, U.S. Patent No. 5,707,796 describes the use of SELEX™ in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. U.S. Patent No. 5,763,177 describes SELEX™ based methods for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. U.S. Patent No. 5,567,588 and U.S. Patent No. 5,861,254 describe SELEX™ based methods which achieve highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. U.S. Patent No. 5,496,938 describes methods for obtaining improved nucleic acid ligands after the SELEX™ process has been performed. U.S. Patent No. 5,705,337 describes methods for covalently linking a ligand to its target.

[0094] SELEX™ can also be used to obtain nucleic acid ligands that bind to more than one site on the target molecule, and to obtain nucleic acid ligands that include non-nucleic acid species that bind to specific sites on the target. SELEX™ provides means for isolating and identifying nucleic acid ligands which bind to any envisionable target, including large and small biomolecules such as nucleic acid-binding proteins and proteins not known to bind nucleic acids as part of their biological function as well as cofactors and other small molecules. For example, U.S. Patent No. 5,580,737 discloses nucleic acid sequences identified through SELEX™ which are capable of binding with high affinity to caffeine and the closely related analog, theophylline.

[0095] Counter-SELEX™ is a method for improving the specificity of nucleic acid ligands to a target molecule by eliminating nucleic acid ligand sequences with cross-reactivity to one or more non-target molecules. Counter-SELEX™ is comprised of the steps of: (a) preparing a candidate mixture of nucleic acids; (b) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate

mixture may be partitioned from the remainder of the candidate mixture; (c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; (d) dissociating the increased affinity nucleic acids from the target; (e) contacting the increased affinity nucleic acids with one or more non-target molecules such that nucleic acid ligands with specific affinity for the non-target molecule(s) are removed; and (f) amplifying the nucleic acids with specific affinity only to the target molecule to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity and specificity for binding to the target molecule. As described above for SELEX™ cycles of selection and amplification are repeated as necessary until a desired goal is achieved.

[0096] One potential problem encountered in the use of nucleic acids as therapeutics and vaccines is that oligonucleotides in their phosphodiester form may be quickly degraded in body fluids by intracellular and extracellular enzymes such as endonucleases and exonucleases before the desired effect is manifest. The SELEX™ method thus encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the sugar and/or phosphate and/or base positions. SELEX™-identified nucleic acid ligands containing modified nucleotides are described, *e.g.*, in U.S. Patent No. 5,660,985, which describes oligonucleotides containing nucleotide derivatives chemically modified at the 2' position of ribose, 5 position of pyrimidines, and 8 position of purines, U.S. Patent No. 5,756,703 which describes oligonucleotides containing various 2'-modified pyrimidines, and U.S. Patent No. 5,580,737 which describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH₂), 2'-fluoro (2'-F), and/or 2'-OMe substituents.

[0097] Modifications of the nucleic acid ligands contemplated in this invention include, but are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and fluxionality to the nucleic acid ligand bases or to the nucleic acid ligand as a whole. Modifications to generate oligonucleotide populations which are resistant to nucleases can also include one or more substitute internucleotide linkages, altered sugars, altered bases, or

combinations thereof. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil, backbone modifications, phosphorothioate or alkyl phosphate modifications, methylations, and unusual base-pairing combinations such as the isobases isocytidine and isoguanidine. Modifications can also include 3' and 5' modifications such as capping.

[0098] In one embodiment, oligonucleotides are provided in which the P(O)O group is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), P(O)NR₂ ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal") or 3'-amine (-NH-CH₂-CH₂-), wherein each R or R' is independently H or substituted or unsubstituted alkyl. Linkage groups can be attached to adjacent nucleotides through an -O-, -N-, or -S- linkage. Not all linkages in the oligonucleotide are required to be identical. As used herein, the term phosphorothioate encompasses one or more non-bridging oxygen atoms in a phosphodiester bond replaced by one or more sulfur atoms

[0099] In further embodiments, the oligonucleotides comprise modified sugar groups, for example, one or more of the hydroxyl groups is replaced with halogen, aliphatic groups, or functionalized as ethers or amines. In one embodiment, the 2'-position of the furanose residue is substituted by any of an O-methyl, O-alkyl, O-allyl, S-alkyl, S-allyl, or halo group. Methods of synthesis of 2'-modified sugars are described, *e.g.*, in Sproat, *et al.*, Nucl. Acid Res. 19:733-738 (1991); Cotten, *et al.*, Nucl. Acid Res. 19:2629-2635 (1991); and Hobbs, *et al.*, Biochemistry 12:5138-5145 (1973). Other modifications are known to one of ordinary skill in the art. Such modifications may be pre-SELEX[™] process modifications or post-SELEX[™] process modifications (modification of previously identified unmodified ligands) or may be made by incorporation into the SELEX[™] process.

[00100] Pre-SELEX[™] process modifications or those made by incorporation into the SELEX process yield nucleic acid ligands with both specificity for their SELEX[™] target and improved stability, *e.g.*, *in vivo* stability. Post-SELEX[™] process modifications made to nucleic acid ligands may result in improved stability, *e.g.*, *in vivo* stability without adversely affecting the binding capacity of the nucleic acid ligand.

[00101] The SELEX™ method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in U.S. Patent No. 5,637,459 and U.S. Patent No. 5,683,867. The SELEX™ method further encompasses combining selected nucleic acid ligands with lipophilic or non-immunogenic high molecular weight compounds in a diagnostic or therapeutic complex, as described, *e.g.*, in U.S. Patent No. 6,011,020, U.S. Patent No. 6,051,698, and PCT Publication No. WO 98/18480. These patents and applications teach the combination of a broad array of shapes and other properties, with the efficient amplification and replication properties of oligonucleotides, and with the desirable properties of other molecules.

[00102] The identification of nucleic acid ligands to small, flexible peptides via the SELEX™ method has also been explored. Small peptides have flexible structures and usually exist in solution in an equilibrium of multiple conformers, and thus it was initially thought that binding affinities may be limited by the conformational entropy lost upon binding a flexible peptide. However, the feasibility of identifying nucleic acid ligands to small peptides in solution was demonstrated in U.S. Patent No. 5,648,214 in which high affinity RNA nucleic acid ligands to substance P, an 11 amino acid peptide, were identified.

[00103] The aptamers with specificity and binding affinity to the target(s) of the present invention are typically selected by the SELEX™ process as described herein. As part of the SELEX™ process, the sequences selected to bind to the target are then optionally minimized to determine the minimal sequence having the desired binding affinity. The selected aptamer sequences and/or the minimized aptamer sequences are optionally optimized by performing random or directed mutagenesis of the sequence to increase binding affinity or alternatively to determine which positions in the sequence are essential for binding activity. For example, a “doped reselections” may be used to explore the sequence requirements within an aptamer. During doped reselection, selections are carried out with a synthetic, degenerate pool that has been designed based on a single sequence. The level of degeneracy usually varies from 70% to 85% wild type nucleotide. In general, neutral mutations are observed following doped reselection but in some cases sequence changes can result in improvements in affinity. Additionally, selections can be performed with sequences incorporating modified sequences to stabilize the aptamer molecules against degradation *in vivo*.

2'MODIFIED SELEX™

[00104] In order for an aptamer to be suitable for use as a therapeutic, it is preferably inexpensive to synthesize, safe and stable *in vivo*. Wild-type RNA and DNA aptamers are typically not stable *in vivo* because of their susceptibility to degradation by nucleases. Resistance to nuclease degradation can be greatly increased, if desired, by the incorporation of modifying groups at the 2'-position.

[00105] 2'-fluoro and 2'-amino groups have been successfully incorporated into oligonucleotide libraries from which aptamers have been subsequently selected. However, these modifications greatly increase the cost of synthesis of the resultant aptamer, and may introduce safety concerns in some cases because of the possibility that the modified nucleotides could be recycled into host DNA by degradation of the modified oligonucleotides and subsequent use of the nucleotides as substrates for DNA synthesis.

[00106] Aptamers that contain 2'-O-methyl ("2'-OMe") nucleotides, as provided in some embodiments herein, overcome many of these drawbacks. Oligonucleotides containing 2'-OMe nucleotides are nuclease-resistant and inexpensive to synthesize. Although 2'-OMe nucleotides are ubiquitous in biological systems, natural polymerases do not accept 2'-OMe NTPs as substrates under physiological conditions, thus there are no safety concerns over the recycling of 2'-OMe nucleotides into host DNA. The SELEX™ methods used to generate 2'-modified aptamers is described, *e.g.*, in U.S. Provisional Patent Application Serial No. 60/430,761, filed December 3, 2002, U.S. Provisional Patent Application Serial No. 60/487,474, filed July 15, 2003, U.S. Provisional Patent Application Serial No. 60/517,039, filed November 4, 2003, U.S. Patent Application No. 10/729,581, filed December 3, 2003, U.S. Patent Application No. 10/873,856 filed June 21, 2004, entitled "Method for *in vitro* Selection of 2'-OMe Substituted Nucleic Acids", and U.S. Provisional Patent Application Serial No. 60/696,295, filed June 30, 2005, entitled "Improved Materials and Methods for the Generation of Fully 2'-Modified Containing Nucleic Acid Transcripts", each of which is herein incorporated by reference in its entirety.

[00107] The present invention includes aptamers that bind to and modulate the function of von Willebrand Factor which contain modified nucleotides (*e.g.*, nucleotides which have a modification at the 2' position) to make the oligonucleotide more stable than the unmodified

oligonucleotide to enzymatic and chemical degradation as well as thermal and physical degradation. Although there are several examples of 2'-OMe containing aptamers in the literature (see, *e.g.*, Ruckman *et al.*, J.Biol.Chem, 1998 273, 20556-20567-695) these were generated by the *in vitro* selection of libraries of modified transcripts in which the C and U residues were 2'-fluoro (2'-F) substituted and the A and G residues were 2'-OH. Once functional sequences were identified then each A and G residue was tested for tolerance to 2'-OMe substitution, and the aptamer was re-synthesized having all A and G residues which tolerated 2'-OMe substitution as 2'-OMe residues. Most of the A and G residues of aptamers generated in this two-step fashion tolerate substitution with 2'-OMe residues, although, on average, approximately 20% do not. Consequently, aptamers generated using this method tend to contain from two to four 2'-OH residues, and stability and cost of synthesis are compromised as a result. By incorporating modified nucleotides into the transcription reaction which generate stabilized oligonucleotides used in oligonucleotide libraries from which aptamers are selected and enriched by SELEX[™] (and/or any of its variations and improvements, including those described herein), the methods of the present invention eliminate the need for stabilizing the selected aptamer oligonucleotides (*e.g.*, by resynthesizing the aptamer oligonucleotides with modified nucleotides).

[00108] In one embodiment, the present invention provides aptamers comprising combinations of 2'-OH, 2'-F, 2'-deoxy, and 2'-OMe modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides. In another embodiment, the present invention provides aptamers comprising combinations of 2'-OH, 2'-F, 2'-deoxy, 2'-OMe, 2'-NH₂, and 2'-methoxyethyl modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides. In another embodiment, the present invention provides aptamers comprising 5⁶ combinations of 2'-OH, 2'-F, 2'-deoxy, 2'-OMe, 2'-NH₂, and 2'-methoxyethyl modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides.

2'- modified aptamers of some embodiments of the invention are created using modified polymerases, *e.g.*, a modified T7 polymerase, having a rate of incorporation of modified nucleotides having bulky substituents at the furanose 2' position that is higher than that of wild-type polymerases. For example, a single mutant T7 polymerase (Y639F) in which the tyrosine residue at position 639 has been changed to phenylalanine readily utilizes 2'deoxy,

2'-amino-, and 2'-fluoro- nucleotide triphosphates (NTPs) as substrates and has been widely used to synthesize modified RNAs for a variety of applications. However, this mutant T7 polymerase reportedly can not readily utilize (*i.e.*, incorporate) NTPs with bulky 2'-substituents such as 2'-OMe or 2'-azido (2'-N₃) substituents. For incorporation of bulky 2'-substituents, a double T7 polymerase mutant (Y639F/H784A) having the histidine at position 784 changed to an alanine residue in addition to the Y639F mutation has been described and has been used in limited circumstances to incorporate modified pyrimidine NTPs. See Padilla, R. and Sousa, R., *Nucleic Acids Res.*, 2002, 30(24): 138. A Y639F/H784A/K378R mutant T7 RNA polymerase has been used in limited circumstances to incorporate modified purine and pyrimidine NTPs, *e.g.*, 2'-OMe NTPs, but requires a spike of 2'-OH GTP for transcription. See Burmeister *et.al.*, *Chemistry and Biology*, 2005, 12: 25-33. A single mutant T7 polymerase (H784A) having the histidine at position 784 changed to an alanine residue has also been described. Padilla *et al.*, *Nucleic Acids Research*, 2002, 30: 138. In both the Y639F/H784A double mutant and H784A single mutant T7 polymerases, the change to a smaller amino acid residue such as alanine allows for the incorporation of bulkier nucleotide substrates, *e.g.*, 2'-O methyl substituted nucleotides. See Chelliserry, K. and Ellington, A.D., *Nature Biotech*, 2004, 9:1155-60. Additional T7 RNA polymerase have been described with mutations in the active site of the T7 RNA polymerase which more readily incorporate bulky 2'-modified substrates, *e.g.* a single T7 mutant RNA polymerase having the tyrosine residue at position 639 changed to a leucine (Y639L). However activity is often sacrificed for increased substrate specificity conferred by such mutations, leading to low transcript yields. See Padilla R and Sousa, R., *Nucleic Acids Res.*, 1999, 27(6): 1561.

[00109] Generally, it has been found that under the conditions disclosed herein, the Y639F single mutant can be used for the incorporation of all 2'-OMe substituted NTPs except GTP and the Y639F/H784A, Y639F/H784A/K378R, Y639L/H784A, and Y639L/H784A/K378R mutant T7 RNA polymerases can be used for the incorporation of all 2'-OMe substituted NTPs including GTP. It is expected that the H784A single mutant possesses properties similar to the Y639F and the Y639F/H784A mutants when used under the conditions disclosed herein.

2'-modified oligonucleotides may be synthesized entirely of modified nucleotides, or with a subset of modified nucleotides. All nucleotides may be modified, and all may contain the same modification. All nucleotides may be modified, but contain different modifications, e.g., all nucleotides containing the same base may have one type of modification, while nucleotides containing other bases may have different types of modification. All purine nucleotides may have one type of modification (or are unmodified), while all pyrimidine nucleotides have another, different type of modification (or are unmodified). In this way, transcripts, or libraries of transcripts are generated using any combination of modifications, including for example, ribonucleotides (2'-OH), deoxyribonucleotides (2'-deoxy), 2'-F, and 2'-OMe nucleotides. A transcription mixture containing 2'-OMe C and U and 2'-OH A and G is referred to as a "rRmY" mixture and aptamers selected therefrom are referred to as "rRmY" aptamers. A transcription mixture containing deoxy A and G and 2'-OMe U and C is referred to as a "dRmY" mixture and aptamers selected therefrom are referred to as "dRmY" aptamers. A transcription mixture containing 2'-OMe A, C, and U, and 2'-OH G is referred to as a "rGmH" mixture and aptamers selected therefrom are referred to as "rGmH" aptamers. A transcription mixture alternately containing 2'-OMe A, C, U and G and 2'-OMe A, U and C and 2'-F G is referred to as a "alternating mixture" and aptamers selected therefrom are referred to as "alternating mixture" aptamers. A transcription mixture containing 2'-OMe A, U, C, and G, where up to 10% of the G's are ribonucleotides is referred to as a "r/mGmH" mixture and aptamers selected therefrom are referred to as "r/mGmH" aptamers. A transcription mixture containing 2'-OMe A, U, and C, and 2'-F G is referred to as a "fGmH" mixture and aptamers selected therefrom are referred to as "fGmH" aptamers. A transcription mixture containing 2'-OMe A, U, and C, and deoxy G is referred to as a "dGmH" mixture and aptamers selected therefrom are referred to as "dGmH" aptamers. A transcription mixture containing deoxy A, and 2'-OMe C, G and U is referred to as a "dAmB" mixture and aptamers selected therefrom are referred to as "dAmB" aptamers, and a transcription mixture containing all 2'-OH nucleotides is referred to as a "rN" mixture and aptamers selected therefrom are referred to as "rN", "rRrY", or "RNA" aptamers. A transcription mixture containing 2'-OH adenosine triphosphate and guanosine triphosphate and deoxy cytidine triphosphate and thymidine triphosphate is referred to as a rRdY mixture and aptamers selected therefrom are referred to as "rRdY" aptamers. A "mRmY" aptamer is

one containing only 2'-OMe nucleotides except for the starting nucleotide which is 2'-hydroxy.

[00110] A preferred embodiment includes any combination of 2'-OH, 2'-deoxy and 2'-OMe nucleotides. Another embodiment includes any combination of 2'-deoxy and 2'-OMe nucleotides. Yet another embodiment includes any combination of 2'-deoxy and 2'-OMe nucleotides in which the pyrimidines are 2'-OMe (such as dRmY, mRmY or dGmH).

[00111] Incorporation of modified nucleotides into the aptamers of the invention may be accomplished before (pre-) the selection process (*e.g.*, a pre-SELEX™ process modification). Optionally, aptamers of the invention in which modified nucleotides have been incorporated by pre-SELEX™ process modification can be further modified by a post-SELEX™ modification process (*i.e.*, a post-SELEX™ process modification after a pre-SELEX™ modification). Pre-SELEX™ process modifications yield modified nucleic acid ligands with specificity for the SELEX™ target and also improved *in vivo* stability. Post-SELEX™ process modifications, *i.e.*, modification (*e.g.*, truncation, deletion, substitution or additional nucleotide modifications of previously identified ligands having nucleotides incorporated by pre-SELEX™ process modification) can result in a further improvement of *in vivo* stability without adversely affecting the binding capacity of the nucleic acid ligand having nucleotides incorporated by pre-SELEX™ process modification.

[00112] To generate pools of 2'-modified (*e.g.*, 2'-OMe) RNA transcripts in conditions under which a polymerase accepts 2'-modified NTPs the Y693F, Y693F/K378R, Y693F/H784A, Y693F/H784A/K378R, Y693L/H784A, Y693L/H784A/K378R Y639L, or the Y639L/K378Rmutant T7 RNA polymerases can be used. A preferred polymerase is the Y639L/H784A mutant T7 RNA polymerase. Another preferred polymerase is the Y639L/H784A/K378R mutant T7 RNA polymerase. Other T7 RNA polymerases, particularly those that exhibit a high tolerance for bulky 2'-substituents, may also be used in the present invention. When used in a template-directed polymerization using the conditions disclosed herein, the Y639L/H784A or the Y639L/H784A/K378R mutant T7 RNA polymerase can be used for the incorporation of all 2'-OMe NTPs, including GTP, with higher transcript yields than achieved by using the Y639F, Y639F/K378R, Y639F/H784A, Y639F/H784A/K378R, Y639L, or the Y639L/K378R mutant T7 RNA polymerases. The Y639L/H784A and

Y639L/H784A/K378R mutant T7 RNA polymerases can be used with but does not require 2'-OH GTP to achieve high yields of 2'-modified, *e.g.*, 2'-OMe containing oligonucleotides.

[00113] A number of factors have been determined to be important for the transcription conditions useful in the methods disclosed herein. For example, increases in the yields of modified transcript are observed when a leader sequence is incorporated into the 5' end of the DNA transcription template. The leader sequence is typically 6-15 nucleotides long, and may be composed of all purines, or a mixture of purine and pyrimidine nucleotides.

[00114] Transcription can be divided into two phases: the first phase is initiation, during which an NTP is added to the 3'-hydroxyl end of GTP (or another substituted guanosine) to yield a dinucleotide which is then extended by about 10-12 nucleotides; the second phase is elongation, during which transcription proceeds beyond the addition of the first about 10-12 nucleotides. It has been found that small amounts of 2'-OH GTP added to a transcription mixture containing an excess of 2'-OMe GTP are sufficient to enable the polymerase to initiate transcription using 2'-OH GTP, but once transcription enters the elongation phase the reduced discrimination between 2'-OMe and 2'-OH GTP, and the excess of 2'-OMe GTP over 2'-OH GTP allows the incorporation of principally the 2'-OMe GTP.

[00115] Another important factor in the incorporation of 2'-OMe substituted nucleotides into transcripts is the use of both divalent magnesium and manganese in the transcription mixture. Different combinations of concentrations of magnesium chloride and manganese chloride have been found to affect yields of 2'-O-methylated transcripts, the optimum concentration of the magnesium and manganese chloride being dependent on the concentration in the transcription reaction mixture of NTPs which complex divalent metal ions. To obtain the greatest yields of maximally 2'-O-methylated transcripts (*i.e.*, all 2'-OMe A, C, and U and about 90% of G nucleotides), concentrations of approximately 5 mM magnesium chloride and 1.5 mM manganese chloride are preferred when each NTP is present at a concentration of 0.5 mM. When the concentration of each NTP is 1.0 mM, concentrations of approximately 6.5 mM magnesium chloride and 2.0 mM manganese chloride are preferred. When the concentration of each NTP is 2.0 mM, concentrations of approximately 9.5 mM magnesium chloride and 3.0 mM manganese chloride are preferred.

In any case, departures from these concentrations of up to two-fold still give significant amounts of modified transcripts.

[00116] Priming transcription with GMP or guanosine, or another non-2'-OMe non-triphosphate is also important. This effect results from the specificity of the polymerase for the initiating nucleotide. As a result, the 5'-terminal nucleotide of any transcript generated in this fashion is likely to be 2'-OH G. The preferred concentration of GMP (or guanosine) is 0.5 mM and even more preferably 1 mM. It has also been found that including PEG, preferably PEG-8000, in the transcription reaction is useful to maximize incorporation of modified nucleotides.

[00117] For maximum incorporation of 2'-OMe ATP (100%), UTP (100%), CTP(100%) and GTP (~90%) ("r/mGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 5 mM (6.5 mM where the concentration of each 2'-OMe NTP is 1.0 mM), MnCl₂ 1.5 mM (2.0 mM where the concentration of each 2'-OMe NTP is 1.0 mM), 2'-OMe NTP (each) 500 μM (more preferably, 1.0 mM), 2'-OH GTP 30 μM, 2'-OH GMP 500 μM, pH 7.5, Y639F/H784A T7 RNA Polymerase 200 nM, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long. As used herein, one unit of the Y639F/H784A mutant T7 RNA polymerase (or any other mutant T7 RNA polymerase specified herein) is defined as the amount of enzyme required to incorporate 1 nmole of 2'-OMe NTPs into transcripts under the r/mGmH conditions. As used herein, one unit of inorganic pyrophosphatase is defined as the amount of enzyme that will liberate 1.0 mole of inorganic orthophosphate per minute at pH 7.2 and 25 °C.

[00118] For maximum incorporation (100%) of 2'-OMe ATP, UTP and CTP ("rGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 5 mM (9.5 mM where the concentration of each 2'-OMe NTP is 2.0 mM), MnCl₂ 1.5 mM (3.0 mM where the concentration of each 2'-OMe NTP is 2.0 mM), 2'-OMe NTP (each) 500 μM (more preferably, 2.0 mM), pH 7.5, Y639F T7 RNA Polymerase 200 nM, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

[00119] For maximum incorporation of 2'-OMe ATP (100%), 2'-OMe UTP (100%), 2'-OMe CTP (100%) and 2'-OMe GTP (100%) ("mRmY") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 8 mM, MnCl₂ 2.5 mM, 2'-OMe NTP (each) 1.5 mM, 2'-OH GMP 1 mM, pH 7.5, Y639L/H784A/K378R mutant T7 RNA Polymerase 200nM, inorganic pyrophosphatase 5 units/ml, and a leader sequence that increases the transcription yield under the derived transcription conditions. In one embodiment, the leader sequence is an all purine leader sequence. In another embodiment, the leader sequence is a mixture of purines and pyrimidines. As used herein, one unit of inorganic pyrophosphatase is defined as the amount of enzyme that will liberate 1.0 mole of inorganic orthophosphate per minute at pH 7.2 and 25 °C.

[00120] For maximum incorporation (100%) of 2'-OMe UTP and CTP ("rRmY") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 5 mM (9.5 mM where the concentration of each 2'-OMe NTP is 2.0 mM), MnCl₂ 1.5 mM (3.0 mM where the concentration of each 2'-OMe NTP is 2.0 mM), 2'-OMe NTP (each) 500μM (more preferably, 2.0 mM), pH 7.5, Y639F/H784A T7 RNA Polymerase 200 nM, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

[00121] For maximum incorporation (100%) of deoxy ATP and GTP and 2'-OMe UTP and CTP ("dRmY") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermine 2 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 9.5 mM, MnCl₂ 3.0 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 200 nM, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

[00122] For maximum incorporation (100%) of 2'-OMe ATP, UTP and CTP and 2'-F GTP ("fGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 9.5 mM, MnCl₂ 3.0 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 200 nM, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

[00123] For maximum incorporation (100%) of deoxy ATP and 2'-OMe UTP, GTP and CTP ("dAmB") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), MgCl₂ 9.5 mM, MnCl₂ 3.0 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 200 nM, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

[00124] For each of the above (a) transcription is preferably performed at a temperature of from about 20 °C to about 50 °C, preferably from about 30 °C to 45 °C, and more preferably at about 37 °C for a period of at least two hours and (b) 50-300 nM of a double stranded DNA transcription template is used (200 nM template is used in round 1 to increase diversity (300 nM template is used in dRmY transcriptions)), and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein, is used). The preferred DNA transcription templates are described below (where ARC254 and ARC256 transcribe under all 2'-OMe conditions and ARC255 transcribes under rRmY conditions).

SEQ ID NO: 1

5'-CATCGATGCTAGTCGTAACGATCCNNCGAGAACGTTCTCTCCTCTCCCTATA
GTGAGTCGTATTA-3'

SEQ ID NO: 2

5'-CATGCATCGCGACTGACTAGCCGNNGTAGAACGTTCTCTCCTCTCCCTATAG
TGAGTCGTATTA-3'

SEQ ID NO 3:

5'-CATCGATCGATCGACAGCGNNGTAGAACGTTCTCTCCTCTCCCTATAG
TGAGTCGTATTA-3'

[00125] Under rN transcription conditions of the present invention, the transcription reaction mixture comprises 2'-OH adenosine triphosphates (ATP), 2'-OH guanosine triphosphates (GTP), 2'-OH cytidine triphosphates (CTP), and 2'-OH uridine triphosphates (UTP). The modified oligonucleotides produced using the rN transcription mixtures of the present invention comprise substantially all 2'-OH adenosine, 2'-OH guanosine, 2'-OH cytidine, and 2'-OH uridine. In a preferred embodiment of rN transcription, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2'-OH adenosine, at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-OH cytidine, and at least 80% of all

uridine nucleotides are 2'-OH uridine. In a more preferred embodiment of rN transcription, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 90% of all adenosine nucleotides are 2'-OH adenosine, at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-OH cytidine, and at least 90% of all uridine nucleotides are 2'-OH uridine. In a most preferred embodiment of rN transcription, the modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2'-OH adenosine, 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-OH cytidine, and 100% of all uridine nucleotides are 2'-OH uridine.

[00126] Under rRmY transcription conditions of the present invention, the transcription reaction mixture comprises 2'-OH adenosine triphosphates, 2'-OH guanosine triphosphates, 2'-OMe cytidine triphosphates, and 2'-OMe uridine triphosphates. The modified oligonucleotides produced using the rRmY transcription mixtures of the present invention comprise substantially all 2'-OH adenosine, 2'-OH guanosine, 2'-OMe cytidine and 2'-OMe uridine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2'-OH adenosine, at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-OMe cytidine and at least 80% of all uridine nucleotides are 2'-OMe uridine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-OH adenosine, at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-OMe cytidine and at least 90% of all uridine nucleotides are 2'-OMe uridine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2'-OH adenosine, 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-OMe cytidine and 100% of all uridine nucleotides are 2'-OMe uridine.

[00127] Under dRmY transcription conditions of the present invention, the transcription reaction mixture comprises 2'-deoxy adenosine triphosphates, 2'-deoxy guanosine triphosphates, 2'-O-methyl cytidine triphosphates, and 2'-O-methyl uridine triphosphates.

The modified oligonucleotides produced using the dRmY transcription conditions of the present invention comprise substantially all 2'-deoxy adenosine, 2'-deoxy guanosine, 2'-O-methyl cytidine, and 2'-O-methyl uridine. In a preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 80% of all adenosine nucleotides are 2'-deoxy adenosine, at least 80% of all guanosine nucleotides are 2'-deoxy guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In a more preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 90% of all adenosine nucleotides are 2'-deoxy adenosine, at least 90% of all guanosine nucleotides are 2'-deoxy guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 90% of all uridine nucleotides are 2'-O-methyl uridine. In a most preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2'-deoxy adenosine, 100% of all guanosine nucleotides are 2'-deoxy guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, and 100% of all uridine nucleotides are 2'-O-methyl uridine.

[00128] Under rGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2'-OH guanosine triphosphates, 2'-O-methyl cytidine triphosphates, 2'-O-methyl uridine triphosphates, and 2'-O-methyl adenosine triphosphates. The modified oligonucleotides produced using the rGmH transcription mixtures of the present invention comprise substantially all 2'-OH guanosine, 2'-O-methyl cytidine, 2'-O-methyl uridine, and 2'-O-methyl adenosine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all guanosine

nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all uridine nucleotides are 2'-O-methyl uridine, and 100% of all adenosine nucleotides are 2'-O-methyl adenosine.

[00129] Under r/mGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2'-O-methyl adenosine triphosphate, 2'-O-methyl cytidine triphosphate, 2'-O-methyl guanosine triphosphate, 2'-O-methyl uridine triphosphate and 2'-OH guanosine triphosphate. The resulting modified oligonucleotides produced using the r/mGmH transcription mixtures of the present invention comprise substantially all 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, wherein the population of guanosine nucleotides has a maximum of about 10% 2'-OH guanosine. In a preferred embodiment, the resulting r/mGmH modified oligonucleotides of the present invention comprise a sequence where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are 2'-OH guanosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are 2'-OH guanosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are 2'-OH guanosine.

[00130] Under mRmY transcription conditions of the present invention, the transcription mixture comprises only 2'-O-methyl adenosine triphosphate, 2'-O-methyl cytidine triphosphate, 2'-O-methyl guanosine triphosphate, 2'-O-methyl uridine triphosphate.. The resulting modified oligonucleotides produced using the mRmY transcription mixture of the

present invention comprise a sequence where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine.

[00131] Under fGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2'-O-methyl adenosine triphosphates, 2'-O-methyl uridine triphosphates, 2'-O-methyl cytidine triphosphates, and 2'-F guanosine triphosphates. The modified oligonucleotides produced using the fGmH transcription conditions of the present invention comprise substantially all 2'-O-methyl adenosine, 2'-O-methyl uridine, 2'-O-methyl cytidine, and 2'-F guanosine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 80% of all guanosine nucleotides are 2'-F guanosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 90% of all guanosine nucleotides are 2'-F guanosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all uridine nucleotides are 2'-O-methyl uridine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, and 100% of all guanosine nucleotides are 2'-F guanosine.

[00132] Under dAmB transcription conditions of the present invention, the transcription reaction mixture comprises 2'-deoxy adenosine triphosphates, 2'-O-methyl cytidine triphosphates, 2'-O-methyl guanosine triphosphates, and 2'-O-methyl uridine triphosphates. The modified oligonucleotides produced using the dAmB transcription mixtures of the present invention comprise substantially all 2'-deoxy adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2'-deoxy adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least

80% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-deoxy adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 90% of all uridine nucleotides are 2'-O-methyl uridine. In a most preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2'-deoxy adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine.

[00133] In each case, the transcription products can then be used for input into the SELEX[™] process to identify aptamers and/or to determine a conserved sequences that has binding specificity to a given target. The resulting sequences are already partially stabilized, eliminating this step from the post-SELEX[™] process to arrive at an optimized aptamer sequence and giving a more highly stabilized aptamer as a result. Another advantage of the 2'-OMe SELEX[™] process is that the resulting sequences are likely to have fewer 2'-OH nucleotides required in the sequence, possibly none. To the extent 2'OH nucleotides remain they may be removed by performing post-SELEX[™] modifications.

[00134] As described below, lower but still useful yields of transcripts fully incorporating 2' substituted nucleotides can be obtained under conditions other than the optimized conditions described above. For example, variations to the above transcription conditions include:

[00135] The HEPES buffer concentration can range from 0 to 1 M. The present invention also contemplates the use of other buffering agents having a pKa between 5 and 10 including, for example, Tris-hydroxymethyl-aminomethane.

[00136] The DTT concentration can range from 0 to 400 mM. The methods of the present invention also provide for the use of other reducing agents including, for example, mercaptoethanol.

[00137] The spermidine and/or spermine concentration can range from 0 to 20 mM.

[00138] The PEG-8000 concentration can range from 0 to 50 % (w/v). The methods of the present invention also provide for the use of other hydrophilic polymer including, for example, other molecular weight PEG or other polyalkylene glycols.

[00139] The Triton X-100 concentration can range from 0 to 0.1% (w/v). The methods of the present invention also provide for the use of other non-ionic detergents including, for example, other detergents, including other Triton-X detergents.

[00140] The MgCl_2 concentration can range from 0.5 mM to 50 mM. The MnCl_2 concentration can range from 0.15 mM to 15 mM. Both MgCl_2 and MnCl_2 must be present within the ranges described and in a preferred embodiment are present in about a 10 to about 3 ratio of MgCl_2 : MnCl_2 , preferably, the ratio is about 3-5:1, more preferably, the ratio is about 3-4:1.

[00141] The 2'-OMe NTP concentration (each NTP) can range from 5 μM to 5 mM.

[00142] The 2'-OH GTP concentration can range from 0 μM to 300 μM .

[00143] The 2'-OH GMP concentration can range from 0 to 5 mM.

[00144] The pH can range from pH 6 to pH 9. The methods of the present invention can be practiced within the pH range of activity of most polymerases that incorporate modified nucleotides. In addition, the methods of the present invention provide for the optional use of chelating agents in the transcription reaction condition including, for example, EDTA, EGTA, and DTT.

APTAMER MEDICINAL CHEMISTRY

[00145] Aptamer Medicinal Chemistry is an aptamer improvement technique in which sets of variant aptamers are chemically synthesized. These sets of variants typically differ from the parent aptamer by the introduction of a single substituent, and differ from each other by the location of this substituent. These variants are then compared to each other and to the parent. Improvements in characteristics may be profound enough that the inclusion of a single substituent may be all that is necessary to achieve a particular therapeutic criterion.

[00146] Alternatively the information gleaned from the set of single variants may be used to design further sets of variants in which more than one substituent is introduced

simultaneously. In one design strategy, all of the single substituent variants are ranked, the top 4 are chosen and all possible double (6), triple (4) and quadruple (1) combinations of these 4 single substituent variants are synthesized and assayed. In a second design strategy, the best single substituent variant is considered to be the new parent and all possible double substituent variants that include this highest-ranked single substituent variant are synthesized and assayed. Other strategies may be used, and these strategies may be applied repeatedly such that the number of substituents is gradually increased while continuing to identify further-improved variants.

[00147] Aptamer Medicinal Chemistry may be used particularly as a method to explore the local, rather than the global, introduction of substituents. Because aptamers are discovered within libraries that are generated by transcription, any substituents that are introduced during the SELEX™ process must be introduced globally. For example, if it is desired to introduce phosphorothioate linkages between nucleotides then they can only be introduced at every A (or every G, C, T, U etc.) (globally substituted). Aptamers which require phosphorothioates at some As (or some G, C, T, U etc.) (locally substituted) but cannot tolerate it at other As cannot be readily discovered by this process.

[00148] The kinds of substituent that can be utilized by the Aptamer Medicinal Chemistry process are only limited by the ability to generate them as solid-phase synthesis reagents and introduce them into an oligomer synthesis scheme. The process is certainly not limited to nucleotides alone. Aptamer Medicinal Chemistry schemes may include substituents that introduce steric bulk, hydrophobicity, hydrophilicity, lipophilicity, lipophobicity, positive charge, negative charge, neutral charge, zwitterions, polarizability, nuclease-resistance, conformational rigidity, conformational flexibility, protein-binding characteristics, mass etc. Aptamer Medicinal Chemistry schemes may include base-modifications, sugar-modifications or phosphodiester linkage-modifications.

[00149] When considering the kinds of substituents that are likely to be beneficial within the context of a therapeutic aptamer, it may be desirable to introduce substitutions that fall into one or more of the following categories:

- (1) Substituents already present in the body, *e.g.*, 2'-deoxy, 2'-ribo, 2'-O-methyl purines or pyrimidines or 5-methyl cytosine.

- (2) Substituents already part of an approved therapeutic, *e.g.*, phosphorothioate-linked oligonucleotides.
- (3) Substituents that hydrolyze or degrade to one of the above two categories, *e.g.*, methylphosphonate-linked oligonucleotides.

[00150] The vWF aptamers of the invention include aptamers developed through aptamer medicinal chemistry as described herein.

VON WILLEBRAND FACTOR SPECIFIC BINDING APTAMERS

[00151] The materials of the present invention comprise a series of nucleic acid aptamers of 29 to 76 nucleotides in length which bind specifically to von Willebrand Factor. In one embodiment, materials of the present invention comprise a series of nucleic acid aptamers of 29 to 76 nucleotides in length which bind specifically to von Willebrand Factor and which functionally modulate, *e.g.*, block, an activity of von Willebrand Factor *in vivo* and/or cell-based assays.

[00152] Aptamers specifically capable of binding and modulating full length von Willebrand Factor and/or von Willebrand Factor domain A1 are set forth herein. These aptamers provide a low-toxicity, safe, and effective modality of treating and/or preventing cardiovascular diseases or disorders. In one embodiment, the aptamers of the invention are used in a method to treat and/or prevent coronary artery diseases, including any one of the disorders selected from the group consisting of: arterial thrombosis and acute coronary syndromes such as unstable angina and myocardial infarction which are known to be caused by or otherwise associated with von Willebrand Factor mediated platelet aggregation. In particular embodiments, the aptamers of the invention are used in a method to treat and/or prevent coronary artery diseases, including any one of the disorders selected from the group consisting of: arterial thrombosis and acute coronary syndromes such as unstable angina and myocardial infarction which are known to be caused by or otherwise associated with von Willebrand Factor mediated platelet aggregation while minimizing bleeding side effects. In another embodiment, the aptamers of the invention are used in a method to treat and/or prevent peripheral vascular diseases which are known to be caused by or otherwise associated

with von Willebrand Factor mediated platelet aggregation. In a particular embodiment, the aptamers of the invention are used in a method to treat and/or prevent peripheral vascular diseases which are known to be caused by or otherwise associated with von Willebrand Factor mediated platelet aggregation, preferably, while minimizing bleeding side effects. In another embodiment, the aptamers of the invention are used to treat and/or prevent cerebrovascular diseases, including any one of the disorders selected from the group consisting of: transient cerebral ischemic attack, stroke and carotid stenosis which are known to be caused by or otherwise associated with von Willebrand Factor mediated platelet aggregation, preferably, while minimizing bleeding side effects. Further, aptamers of the invention are useful to inhibit von Willebrand Factor mediated platelet aggregation in a subject prior to, during, and/or after a subject has undergone percutaneous coronary intervention including angioplasty, thrombolytic treatment or coronary bypass surgery. Aptamers of the invention are also useful for maintaining blood vessel patency in a subject prior to, during and/or after the subject has undergone coronary bypass surgery. The aptamers of the invention are also useful for treating a patient undergoing dialysis. The aptamers of the invention are also useful for inhibiting von Willebrand Factor mediated thrombosis in a subject, preferably while also minimizing bleeding side effects. The thrombosis to be treated and/or inhibited may be associated with an inflammatory response.

[00153] In one embodiment, the von Willebrand Factor specific binding aptamer for use in therapeutics and/or diagnostics is selected from the group consisting of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635, ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323).

[00154] In another embodiment, von Willebrand Factor specific binding aptamers for use as therapeutics and/or diagnostics include any one of the following sequences: SEQ ID NO 23, SEQ ID NO 44, SEQ ID NO 49, SEQ ID NOS 98-100, SEQ ID NO 106, SEQ ID NO 109, SEQ ID NOS 114 to 115, SEQ ID NO 118, SEQ ID NO 127, SEQ ID NO 134, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 208, and SEQ ID NOS 212 to 214. In some embodiments, von Willebrand Factor specific binding aptamers for use as therapeutics and/or diagnostics include any one of the following sequences: ARC1029 (SEQ ID NO 214), ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284), ARC1368 (SEQ ID NO 291), ARC1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320), ARC 1780 (SEQ ID NO 321), ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323).

[00155] Other aptamers of the invention that bind von Willebrand Factor are described below in Examples 1 and 2.

[00156] These aptamers may include modifications as described herein including, *e.g.*, conjugation to lipophilic or high molecular weight compounds (*e.g.*, PEG), incorporation of a capping moiety, incorporation of modified nucleotides, and phosphate back bone modification (including incorporation of phosphorothioate into the phosphate backbone).

[00157] In one embodiment of the invention an isolated, non-naturally occurring aptamer that binds to von Willebrand Factor is provided. In another embodiment, the aptamer of the invention modulates a function of von Willebrand Factor. In another embodiment, the aptamer of the invention inhibits a function of von Willebrand Factor while in another embodiment the aptamer stimulates a function of von Willebrand Factor. In another embodiment of the invention, the aptamer binds and/or modulates a function of a von Willebrand Factor variant. A von Willebrand Factor variant as used herein encompasses variants that perform essentially the same function as a von Willebrand Factor function, preferably comprises substantially the same structure and in some embodiments comprises at least 70% sequence identity, preferably at least 80% sequence identity, more preferably at

least 90% sequence identity, and more preferably at least 95% sequence identity to the amino acid sequence of human von Willebrand Factor.

[00158] In another embodiment of the invention, the aptamer has substantially the same ability to bind von Willebrand Factor as that of an aptamer comprising any one of SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115, ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635, ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In another embodiment of the invention, the aptamer has substantially the same structure and ability to bind von Willebrand Factor as that of an aptamer comprising any one of SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115, ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635, ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In another embodiment, the aptamers of the invention comprise a sequence according to any one of SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-213, ARC1115, ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID

NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635, ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In another embodiment, the aptamers of the invention comprise a sequence that is at least 80% identical, preferably at least 90% identical and in some embodiments at least 95% identical to a sequence according to any one of SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115, ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635, ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In another embodiment, the aptamers of the invention specifically bind von Willebrand Factor and comprise a sequence of 30 contiguous nucleotides that are identical to 30 contiguous nucleotides in any one of the aptamers selected from the group consisting of: SEQ ID NOS 11 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 165, SEQ ID NO 169, SEQ ID NO 172, SEQ ID NO 174, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115, ARC1172 (SEQ ID NO 222) (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1635, ARC1759 (SEQ ID NO 318), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323). In another embodiment, the aptamers of the invention are used as an active ingredient in pharmaceutical compositions. In another embodiment, the aptamers of the invention or

compositions comprising the aptamers of the invention are used to treat thrombotic disease such as cardiovascular disorders, including acute coronary syndrome; peripheral arterial disease; and cerebrovascular disorders, including stroke. In some embodiments, the aptamers of the invention or compositions comprising the aptamers of the invention are used to treat, prevent or ameliorate a disorder selected from the group consisting of: essential thrombocytopenia; thrombotic thrombocopenic purpura ("TTP"), Type IIb von Willebrand's disease, pseudo von Willebrand disease, peripheral artery disease, *e.g.* peripheral arterial occlusive disease, unstable angina, angina pectoris, arterial thrombosis, atherosclerosis, myocardial infarction, acute coronary syndrome, atrial fibrillation, carotid stenosis, cerebral infarction, cerebral thrombosis, ischemic stroke, and transient cerebral ischemic attack. In some embodiments, the pharmaceutical composition of the invention is administered prior to/during and/or after dialysis, CABG surgery, percutaneous coronary intervention or heart valve replacement.

[00159] In some embodiments, aptamer therapeutics of the present invention have great affinity and specificity to their targets while reducing the deleterious side effects from non-naturally occurring nucleotide substitutions if the aptamer therapeutics break down in the body of patients or subjects. In some embodiments, the therapeutic compositions containing the aptamer therapeutics of the present invention are free of or have a reduced amount of fluorinated nucleotides.

[00160] The aptamers of the present invention can be synthesized using any oligonucleotide synthesis techniques known in the art including solid phase oligonucleotide synthesis techniques (see, *e.g.*, Froehler *et al.*, Nucl. Acid Res. 14:5399-5467 (1986) and Froehler *et al.*, Tet. Lett. 27:5575-5578 (1986)) and solution phase methods such as triester synthesis methods (see, *e.g.*, Sood *et al.*, Nucl. Acid Res. 4:2557 (1977) and Hirose *et al.*, Tet. Lett, 28:2449 (1978)) both of which are well known in the art.

PHARMACEUTICAL COMPOSITIONS

[00161] The invention also includes pharmaceutical compositions containing aptamer molecules that bind to von Willebrand Factor. In some embodiments, the compositions are

suitable for internal use and include an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more pharmaceutically acceptable carriers. The compounds are especially useful in that they have very low, if any toxicity.

[00162] Compositions of the invention can be used to treat or prevent a pathology, such as a disease or disorder, or alleviate the symptoms of such disease or disorder in a patient. For example, compositions of the present invention can be used to treat or prevent a pathology associated with platelet aggregation. In some embodiments, the disease to be treated, prevented or ameliorated is selected from the group consisting of: essential thrombocytopenia, thrombotic thrombocopenic purpura ("TTP"), Type IIb von Willebrand's disease, pseudo von Willebrand disease, peripheral artery disease, *e.g.* peripheral arterial occlusive disease, unstable angina, angina pectoris, arterial thrombosis, atherosclerosis, myocardial infarction, acute coronary syndrome, atrial fibrillation, carotid stenosis, cerebral infarction, cerebral thrombosis, ischemic stroke, and transient cerebral ischemic attack. In some embodiments, the pharmaceutical composition of the invention is administered prior to, during and/or after dialysis, CABG surgery, percutaneous coronary intervention or heart valve replacement.

[00163] Compositions of the invention are useful for administration to a subject suffering from, or predisposed to, a disease or disorder which is related to or derived from a target to which the aptamers of the invention specifically bind.

[00164] Compositions of the invention can be used in a method for treating a patient or subject having a pathology. The method involves administering to the patient or subject an aptamer or a composition comprising aptamers that bind to von Willebrand Factor involved with the pathology, so that binding of the aptamer to the target alters the biological function of von Willebrand Factor, thereby treating the pathology.

[00165] The patient or subject having a pathology, *i.e.*, the patient or subject treated by the methods of this invention can be a mammal, more particularly a vertebrate, or more particularly, a human.

[00166] In practice, the aptamers or their pharmaceutically acceptable salts, are administered in amounts which will be sufficient to exert their desired biological activity, *e.g.*, preventing vWF dependent platelet aggregation.

[00167] One aspect of the invention comprises an aptamer composition of the invention in combination with other treatments for thrombotic related disorders. The aptamer composition of the invention may contain, for example, more than one aptamer, *e.g.* an anti-thrombin aptamer and an anti-vWF aptamer. In some examples, an aptamer composition of the invention, containing one or more aptamers of the invention, is administered in combination with another useful composition such as an anti-inflammatory agent, an immunosuppressant, an antiviral agent, or the like. In general, the currently available dosage forms of the known therapeutic agents for use in such combinations will be suitable.

[00168] "Combination therapy" (or "co-therapy") includes the administration of an aptamer composition of the invention and at least a second agent as part of a specific treatment regimen intended to provide the beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days or weeks depending upon the combination selected).

[00169] "Combination therapy" may, but generally is not, intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present invention. "Combination therapy" is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single capsule having a fixed ratio of each therapeutic agent or in multiple, single capsules for each of the therapeutic agents.

[00170] Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, topical routes, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination selected may be administered by injection while the other therapeutic agents of the combination may be administered topically.

[00171] Alternatively, for example, all therapeutic agents may be administered topically or all therapeutic agents may be administered by injection. The sequence in which the therapeutic agents are administered is not narrowly critical unless noted otherwise. "Combination therapy" also can embrace the administration of the therapeutic agents as described above in further combination with other biologically active ingredients. Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and non-drug treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

[00172] Therapeutic or pharmacological compositions of the present invention will generally comprise an effective amount of the active component(s) of the therapy, dissolved or dispersed in a pharmaceutically acceptable medium. Pharmaceutically acceptable media or carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the therapeutic compositions of the present invention.

[00173] The preparation of pharmaceutical or pharmacological compositions will be known to those of skill in the art in light of the present disclosure. Typically, such compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection; as tablets or other

solids for oral administration; as time release capsules; or in any other form currently used, including eye drops, creams, lotions, salves, inhalants and the like. The use of sterile formulations, such as saline-based washes, by surgeons, physicians or health care workers to treat a particular area in the operating field may also be particularly useful. Compositions may also be delivered via microdevice, microparticle or sponge.

[00174] Upon formulation, therapeutics will be administered in a manner compatible with the dosage formulation, and in such amount as is pharmacologically effective. The formulations are easily administered in a variety of dosage forms. In a preferred embodiment the aptamer of the invention is formulated as an injectable solution described above, but drug release capsules and the like can also be employed.

[00175] In this context, the quantity of active ingredient and volume of composition to be administered depends on the host animal to be treated. Precise amounts of active compound required for administration depend on the judgment of the practitioner and are peculiar to each individual.

[00176] A minimal volume of a composition required to disperse the active compounds is typically utilized. Suitable regimes for administration are also variable, but would be typified by initially administering the compound and monitoring the results and then giving further controlled doses at further intervals. The effects of administration of the anti-vWF aptamer of the invention could be monitored by measuring platelet aggregation formation such as measuring botrocetin induced platelet aggregation ("BIPA") and/or shear force induced hemostatic plug formation using the PFA-100 instrument as described in Example 3 below.

[00177] For instance, for oral administration in the form of a tablet or capsule (*e.g.*, a gelatin capsule), the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, magnesium aluminum silicate, starch paste, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms

include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum starches, agar, alginic acid or its sodium salt, or effervescent mixtures, and the like. Diluents, include, *e.g.*, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine.

[00178] Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. The compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. The compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and typically contain about 0.1 to 75%, preferably about 1 to 50%, of the active ingredient.

[00179] The compounds of the invention can also be administered in such oral dosage forms as timed release and sustained release tablets or capsules, pills, powders, granules, elixirs, tinctures, suspensions, syrups and emulsions.

[00180] Liquid, particularly injectable compositions can, for example, be prepared by dissolving, dispersing, etc. The active compound is dissolved in or mixed with a pharmaceutically pure solvent such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form the injectable solution or suspension. Additionally, solid forms suitable for dissolving in liquid prior to injection can be formulated.

[00181] The compounds of the present invention can be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions.

[00182] Parenteral injectable administration is generally used for subcutaneous, intramuscular or intravenous injections and infusions. Additionally, one approach for parenteral administration employs the implantation of a slow-release or sustained-released

systems, which assures that a constant level of dosage is maintained, according to U.S. Pat. No. 3,710,795, incorporated herein by reference.

[00183] Furthermore, preferred compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, inhalants, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Other preferred topical preparations include creams, ointments, lotions, aerosol sprays and gels, wherein the concentration of active ingredient would typically range from 0.01% to 15%, w/w or w/v.

[00184] For solid compositions, excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound defined above, may be also formulated as suppositories using for example, polyalkylene glycols, for example, propylene glycol, as the carrier. In some embodiments, suppositories are advantageously prepared from fatty emulsions or suspensions.

[00185] The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines. In some embodiments, a film of lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in U.S. Pat. No. 5,262,564. For example, the aptamer molecules described herein can be provided as a complex with a lipophilic compound or non-immunogenic, high molecular weight compound constructed using methods known in the art. Additionally, liposomes may bear aptamers on their surface for targeting and carrying cytotoxic agents internally to mediate cell killing. An example of nucleic-acid associated complexes is provided in U.S. Patent No. 6,011,020.

[00186] The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide-phenol, polyhydroxyethylaspanamidephenol,

or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

[00187] If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and other substances such as for example, sodium acetate, and triethanolamine oleate.

[00188] The dosage regimen utilizing the aptamers is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular aptamer or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[00189] Oral dosages of the present invention, when used for the indicated effects, will range between about 0.05 to 7500 mg/day orally. The compositions are preferably provided in the form of scored tablets containing 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mg of active ingredient. Infused dosages, intranasal dosages and transdermal dosages will range between 0.05 to 7500 mg/day. Subcutaneous, intravenous and intraperitoneal dosages will range between 0.05 to 3800 mg/day.

[00190] Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily.

[00191] Effective plasma levels of the compounds of the present invention range from 0.002 mg/mL to 50 mg/mL. In the dosages of the present invention, mass refers only to the molecular weight of the oligonucleotide portion of the aptamer, irrespective of the mass conferred by PEG conjugation.

MODULATION OF PHARMACOKINETICS AND BIODISTRIBUTION OF APTAMER
THERAPEUTICS

[00192] It is important that the pharmacokinetic properties for all oligonucleotide-based therapeutics, including aptamers, be tailored to match the desired pharmaceutical application. While aptamers directed against extracellular targets do not suffer from difficulties associated with intracellular delivery (as is the case with antisense and RNAi-based therapeutics), such aptamers must still be able to be distributed to target organs and tissues, and remain in the body (unmodified) for a period of time consistent with the desired dosing regimen.

[00193] Thus, the present invention provides materials and methods to affect the pharmacokinetics of aptamer compositions, and, in particular, the ability to tune aptamer pharmacokinetics. The tunability of (*i.e.*, the ability to modulate) aptamer pharmacokinetics is achieved through conjugation of modifying moieties (*e.g.*, PEG polymers) to the aptamer and/or the incorporation of modified nucleotides (*e.g.*, 2'-fluoro or 2'-O-methyl) to alter the chemical composition of the nucleic acid. The ability to tune aptamer pharmacokinetics is used in the improvement of existing therapeutic applications, or alternatively, in the development of new therapeutic applications. For example, in some therapeutic applications, *e.g.*, in anti-neoplastic or acute care settings where rapid drug clearance or turn-off may be desired, it is desirable to decrease the residence times of aptamers in the circulation. Alternatively, in other therapeutic applications, *e.g.*, maintenance therapies where systemic circulation of a therapeutic is desired, it may be desirable to increase the residence times of aptamers in circulation.

[00194] In addition, the tunability of aptamer pharmacokinetics is used to modify the biodistribution of an aptamer therapeutic in a subject. For example, in some therapeutic applications, it may be desirable to alter the biodistribution of an aptamer therapeutic in an effort to target a particular type of tissue or a specific organ (or set of organs). In these applications, the aptamer therapeutic preferentially accumulates in a specific tissue or organ(s). In other therapeutic applications, it may be desirable to target tissues displaying a cellular marker or a symptom associated with a given disease, cellular injury or other abnormal pathology, such that the aptamer therapeutic preferentially accumulates in the affected tissue. For example, as described in copending provisional application United States

Serial No. 60/550790, filed on March 5, 2004, and entitled "Controlled Modulation of the Pharmacokinetics and Biodistribution of Aptamer Therapeutics), PEGylation of an aptamer therapeutic (*e.g.*, PEGylation with a 20 kDa PEG polymer) is used to target inflamed tissues, such that the PEGylated aptamer therapeutic preferentially accumulates in inflamed tissue.

[00195] To determine the pharmacokinetic and biodistribution profiles of aptamer therapeutics (*e.g.*, aptamer conjugates or aptamers having altered chemistries, such as modified nucleotides) a variety of parameters are monitored. Such parameters include, for example, the half-life ($t_{1/2}$), the plasma clearance (CL), the volume of distribution (V_{ss}), the area under the concentration-time curve (AUC), maximum observed serum or plasma concentration (C_{max}), and the mean residence time (MRT) of an aptamer composition. As used herein, the term "AUC" refers to the area under the plot of the plasma concentration of an aptamer therapeutic versus the time after aptamer administration. The AUC value is used to estimate the bioavailability (*i.e.*, the percentage of administered aptamer therapeutic in the circulation after aptamer administration) and/or total clearance (CL) (*i.e.*, the rate at which the aptamer therapeutic is removed from circulation) of a given aptamer therapeutic. The volume of distribution relates the plasma concentration of an aptamer therapeutic to the amount of aptamer present in the body. The larger the V_{ss} , the more an aptamer is found outside of the plasma (*i.e.*, the more extravasation).

[00196] The present invention provides materials and methods to modulate, in a controlled manner, the pharmacokinetics and biodistribution of stabilized aptamer compositions *in vivo* by conjugating an aptamer to a modulating moiety such as a small molecule, peptide, or polymer terminal group, or by incorporating modified nucleotides into an aptamer. As described herein, conjugation of a modifying moiety and/or altering nucleotide(s) chemical composition alter fundamental aspects of aptamer residence time in circulation and distribution to tissues.

[00197] In addition to clearance by nucleases, oligonucleotide therapeutics are subject to elimination *via* renal filtration. As such, a nuclease-resistant oligonucleotide administered intravenously typically exhibits an *in vivo* half-life of <10 min, unless filtration can be blocked. This can be accomplished by either facilitating rapid distribution out of the blood stream into tissues or by increasing the apparent molecular weight of the oligonucleotide

above the effective size cut-off for the glomerulus. Conjugation of small therapeutics to a PEG polymer (PEGylation), described below, can dramatically lengthen residence times of aptamers in circulation, thereby decreasing dosing frequency and enhancing effectiveness against vascular targets.

[00198] Aptamers can be conjugated to a variety of modifying moieties, such as high molecular weight polymers, *e.g.*, PEG; peptides, *e.g.*, Tat (a 13-amino acid fragment of the HIV Tat protein (Vives, *et al.*, (1997), J. Biol. Chem. 272(25): 16010-7)), Ant (a 16-amino acid sequence derived from the third helix of the Drosophila antennapedia homeotic protein (Pietersz, *et al.*, (2001), Vaccine 19(11-12): 1397-405)) and Arg₇ (a short, positively charged cell-permeating peptides composed of polyarginine (Arg₇) (Rothbard, *et al.*, (2000), Nat. Med. 6(11): 1253-7; Rothbard, J *et al.*, (2002), J. Med. Chem. 45(17): 3612-8)); and small molecules, *e.g.*, lipophilic compounds such as cholesterol. Among the various conjugates described herein, *in vivo* properties of aptamers are altered most profoundly by complexation with PEG groups. For example, complexation of a mixed 2'-F and 2'-OMe modified aptamer therapeutic with a 20 kDa PEG polymer hinders renal filtration and promotes aptamer distribution to both healthy and inflamed tissues. Furthermore, the 20 kDa PEG polymer-aptamer conjugate proves nearly as effective as a 40 kDa PEG polymer in preventing renal filtration of aptamers. While one effect of PEGylation is on aptamer clearance, the prolonged systemic exposure afforded by presence of the 20 kDa moiety also facilitates distribution of aptamer to tissues, particularly those of highly perfused organs and those at the site of inflammation. The aptamer-20 kDa PEG polymer conjugate directs aptamer distribution to the site of inflammation, such that the PEGylated aptamer preferentially accumulates in inflamed tissue. In some instances, the 20 kDa PEGylated aptamer conjugate is able to access the interior of cells, such as, for example, kidney cells.

[00199] Modified nucleotides can also be used to modulate the plasma clearance of aptamers. For example, an unconjugated aptamer which incorporates both 2'-F and 2'-OMe stabilizing chemistries, which is typical of current generation aptamers as it exhibits a high degree of nuclease stability *in vitro* and *in vivo*, displays rapid loss from plasma (*i.e.*, rapid plasma clearance) and a rapid distribution into tissues, primarily into the kidney, when compared to unmodified aptamer.

PEG-DERIVATIZED NUCLEIC ACIDS

[00200] As described above, derivatization of nucleic acids with high molecular weight non-immunogenic polymers has the potential to alter the pharmacokinetic and pharmacodynamic properties of nucleic acids making them more effective therapeutic agents. Favorable changes in activity can include increased resistance to degradation by nucleases, decreased filtration through the kidneys, decreased exposure to the immune system, and altered distribution of the therapeutic through the body.

[00201] The aptamer compositions of the invention may be derivatized with polyalkylene glycol (PAG) moieties. Examples of PAG-derivatized nucleic acids are found in United States Patent Application Ser. No. 10/718,833, filed on November 21, 2003, which is herein incorporated by reference in its entirety. Typical polymers used in the invention include poly(ethylene glycol) (PEG), also known as poly(ethylene oxide) (PEO) and polypropylene glycol (including poly isopropylene glycol). Additionally, random or block copolymers of different alkylene oxides (*e.g.*, ethylene oxide and propylene oxide) can be used in many applications. In its most common form, a polyalkylene glycol, such as PEG, is a linear polymer terminated at each end with hydroxyl groups: $\text{HO-CH}_2\text{CH}_2\text{O-(CH}_2\text{CH}_2\text{O)}_n\text{-CH}_2\text{CH}_2\text{-OH}$. This polymer, alpha-, omega-dihydroxypoly(ethylene glycol), can also be represented as HO-PEG-OH, where it is understood that the —PEG— symbol represents the following structural unit: $\text{-CH}_2\text{CH}_2\text{O-(CH}_2\text{CH}_2\text{O)}_n\text{-CH}_2\text{CH}_2\text{-}$ where *n* typically ranges from about 4 to about 10,000.

[00202] As shown, the PEG molecule is di-functional and is sometimes referred to as "PEG diol." The terminal portions of the PEG molecule are relatively non-reactive hydroxyl moieties, the —OH groups, that can be activated, or converted to functional moieties, for attachment of the PEG to other compounds at reactive sites on the compound. Such activated PEG diols are referred to herein as bi-activated PEGs. For example, the terminal moieties of PEG diol have been functionalized as active carbonate ester for selective reaction with amino moieties by substitution of the relatively nonreactive hydroxyl moieties, —OH, with succinimidyl active ester moieties from N-hydroxy succinimide.

[00203] In many applications, it is desirable to cap the PEG molecule on one end with an essentially non-reactive moiety so that the PEG molecule is mono-functional (or mono-activated). In the case of protein therapeutics which generally display multiple reaction sites for activated PEGs, bi-functional activated PEGs lead to extensive cross-linking, yielding poorly functional aggregates. To generate mono-activated PEGs, one hydroxyl moiety on the terminus of the PEG diol molecule typically is substituted with non-reactive methoxy end moiety, -OCH₃. The other, un-capped terminus of the PEG molecule typically is converted to a reactive end moiety that can be activated for attachment at a reactive site on a surface or a molecule such as a protein.

[00204] PAGs are polymers which typically have the properties of solubility in water and in many organic solvents, lack of toxicity, and lack of immunogenicity. One use of PAGs is to covalently attach the polymer to insoluble molecules to make the resulting PAG-molecule "conjugate" soluble. For example, it has been shown that the water-insoluble drug paclitaxel, when coupled to PEG, becomes water-soluble. Greenwald, *et al.*, *J. Org. Chem.*, 60:331-336 (1995). PAG conjugates are often used not only to enhance solubility and stability but also to prolong the blood circulation half-life of molecules.

[00205] Polyalkylated compounds of the invention are typically between 5 and 80 kDa in size however any size can be used, the choice dependent on the aptamer and application. Other PAG compounds of the invention are between 10 and 80 kDa in size. Still other PAG compounds of the invention are between 10 and 60 kDa in size. For example, a PAG polymer may be at least 10, 20, 30, 40, 50, 60, or 80 kDa in size. Such polymers can be linear or branched.

[00206] In contrast to biologically-expressed protein therapeutics, nucleic acid therapeutics are typically chemically synthesized from activated monomer nucleotides. PEG-nucleic acid conjugates may be prepared by incorporating the PEG using the same iterative monomer synthesis. For example, PEGs activated by conversion to a phosphoramidite form can be incorporated into solid-phase oligonucleotide synthesis. Alternatively, oligonucleotide synthesis can be completed with site-specific incorporation of a reactive PEG attachment site. Most commonly this has been accomplished by addition of a free primary amine at the 5'-terminus (incorporated using a modifier phosphoramidite in the last coupling step of solid

phase synthesis). Using this approach, a reactive PEG (*e.g.*, one which is activated so that it will react and form a bond with an amine) is combined with the purified oligonucleotide and the coupling reaction is carried out in solution.

[00207] The ability of PEG conjugation to alter the biodistribution of a therapeutic is related to a number of factors including the apparent size (*e.g.*, as measured in terms of hydrodynamic radius) of the conjugate. Larger conjugates (>10kDa) are known to more effectively block filtration via the kidney and to consequently increase the serum half-life of small macromolecules (*e.g.*, peptides, antisense oligonucleotides). The ability of PEG conjugates to block filtration has been shown to increase with PEG size up to approximately 50 kDa (further increases have minimal beneficial effect as half life becomes defined by macrophage-mediated metabolism rather than elimination via the kidneys).

[00208] Production of high molecular weight PEGs (>10 kDa) can be difficult, inefficient, and expensive. As a route towards the synthesis of high molecular weight PEG-nucleic acid conjugates, previous work has been focused towards the generation of higher molecular weight activated PEGs. One method for generating such molecules involves the formation of a branched activated PEG in which two or more PEGs are attached to a central core carrying the activated group. The terminal portions of these higher molecular weight PEG molecules, *i.e.*, the relatively non-reactive hydroxyl (–OH) moieties, can be activated, or converted to functional moieties, for attachment of one or more of the PEGs to other compounds at reactive sites on the compound. Branched activated PEGs will have more than two termini, and in cases where two or more termini have been activated, such activated higher molecular weight PEG molecules are referred to herein as, multi-activated PEGs. In some cases, not all termini in a branch PEG molecule are activated. In cases where any two termini of a branch PEG molecule are activated, such PEG molecules are referred to as bi-activated PEGs. In some cases where only one terminus in a branch PEG molecule is activated, such PEG molecules are referred to as mono-activated. As an example of this approach, activated PEG prepared by the attachment of two monomethoxy PEGs to a lysine core which is subsequently activated for reaction has been described (Harris *et al.*, Nature, vol.2: 214-221, 2003).

[00209] The present invention provides another cost effective route to the synthesis of high molecular weight PEG-nucleic acid (preferably, aptamer) conjugates including multiply

PEGylated nucleic acids. The present invention also encompasses PEG-linked multimeric oligonucleotides, *e.g.*, dimerized aptamers. The present invention also relates to high molecular weight compositions where a PEG stabilizing moiety is a linker which separates different portions of an aptamer, *e.g.*, the PEG is conjugated within a single aptamer sequence, such that the linear arrangement of the high molecular weight aptamer composition is, *e.g.*, nucleic acid – PEG – nucleic acid (– PEG — nucleic acid)_n where n is greater than or equal to 1.

[00210] High molecular weight compositions of the invention include those having a molecular weight of at least 10 kDa. Compositions typically have a molecular weight between 10 and 80 kDa in size. High molecular weight compositions of the invention are at least 10, 20, 30, 40, 50, 60, or 80 kDa in size.

[00211] A stabilizing moiety is a molecule, or portion of a molecule, which improves pharmacokinetic and pharmacodynamic properties of the high molecular weight aptamer compositions of the invention. In some cases, a stabilizing moiety is a molecule or portion of a molecule which brings two or more aptamers, or aptamer domains, into proximity, or provides decreased overall rotational freedom of the high molecular weight aptamer compositions of the invention. A stabilizing moiety can be a polyalkylene glycol, such a polyethylene glycol, which can be linear or branched, a homopolymer or a heteropolymer. Other stabilizing moieties include polymers such as peptide nucleic acids (PNA). Oligonucleotides can also be stabilizing moieties; such oligonucleotides can include modified nucleotides, and/or modified linkages, such as phosphorothioates. A stabilizing moiety can be an integral part of an aptamer composition, *i.e.*, it is covalently bonded to the aptamer.

[00212] Compositions of the invention include high molecular weight aptamer compositions in which two or more nucleic acid moieties are covalently conjugated to at least one polyalkylene glycol moiety. The polyalkylene glycol moieties serve as stabilizing moieties. In compositions where a polyalkylene glycol moiety is covalently bound at either end to an aptamer, such that the polyalkylene glycol joins the nucleic acid moieties together in one molecule, the polyalkylene glycol is said to be a linking moiety. In such compositions, the primary structure of the covalent molecule includes the linear arrangement nucleic acid-PAG-nucleic acid. One example is a composition having the primary structure nucleic acid-

PEG-nucleic acid. Another example is a linear arrangement of: nucleic acid – PEG – nucleic acid – PEG — nucleic acid.

[00213] To produce the nucleic acid—PEG—nucleic acid conjugate, the nucleic acid is originally synthesized such that it bears a single reactive site (*e.g.*, it is mono-activated). In a preferred embodiment, this reactive site is an amino group introduced at the 5'-terminus by addition of a modifier phosphoramidite as the last step in solid phase synthesis of the oligonucleotide. Following deprotection and purification of the modified oligonucleotide, it is reconstituted at high concentration in a solution that minimizes spontaneous hydrolysis of the activated PEG. In a preferred embodiment, the concentration of oligonucleotide is 1 mM and the reconstituted solution contains 200 mM NaHCO₃-buffer, pH 8.3. Synthesis of the conjugate is initiated by slow, step-wise addition of highly purified bi-functional PEG. In a preferred embodiment, the PEG diol is activated at both ends (bi-activated) by derivatization with succinimidyl propionate. Following reaction, the PEG-nucleic acid conjugate is purified by gel electrophoresis or liquid chromatography to separate fully-, partially-, and un-conjugated species. Multiple PAG molecules concatenated (*e.g.*, as random or block copolymers) or smaller PAG chains can be linked to achieve various lengths (or molecular weights). Non-PAG linkers can be used between PAG chains of varying lengths.

[00214] The 2'-O-methyl, 2'-fluoro and other modified nucleotide modifications stabilize the aptamer against nucleases and increase its half life *in vivo*. The 3'-3'-dT cap also increases exonuclease resistance. See, *e.g.*, U.S. Patents 5,674,685; 5,668,264; 6,207,816; and 6,229,002, each of which is incorporated by reference herein in its entirety.

PAG-DERIVATIZATION OF A REACTIVE NUCLEIC ACID

[00215] High molecular weight PAG-nucleic acid-PAG conjugates can be prepared by reaction of a mono-functional activated PEG with a nucleic acid containing more than one reactive site. In one embodiment, the nucleic acid is bi-reactive, or bi-activated, and contains two reactive sites: a 5'-amino group and a 3'-amino group introduced into the oligonucleotide through conventional phosphoramidite synthesis, for example: 3'-5'-di-PEGylation as illustrated in Figure 2. In alternative embodiments, reactive sites can be introduced at internal positions, using for example, the 5-position of pyrimidines, the 8-position of purines, or the 2'-position of ribose as sites for attachment of primary amines. In such embodiments, the

nucleic acid can have several activated or reactive sites and is said to be multiply activated. Following synthesis and purification, the modified oligonucleotide is combined with the mono-activated PEG under conditions that promote selective reaction with the oligonucleotide reactive sites while minimizing spontaneous hydrolysis. In the preferred embodiment, monomethoxy-PEG is activated with succinimidyl propionate and the coupled reaction is carried out at pH 8.3. To drive synthesis of the bi-substituted PEG, stoichiometric excess PEG is provided relative to the oligonucleotide. Following reaction, the PEG-nucleic acid conjugate is purified by gel electrophoresis or liquid chromatography to separate fully-, partially-, and un-conjugated species.

[00216] The linking domains can also have one or more polyalkylene glycol moieties attached thereto. Such PAGs can be of varying lengths and may be used in appropriate combinations to achieve the desired molecular weight of the composition.

[00217] The effect of a particular linker can be influenced by both its chemical composition and length. A linker that is too long, too short, or forms unfavorable steric and/or ionic interactions with the target will preclude the formation of complex between aptamer and target. A linker, which is longer than necessary to span the distance between nucleic acids, may reduce binding stability by diminishing the effective concentration of the ligand. Thus, it is often necessary to optimize linker compositions and lengths in order to maximize the affinity of an aptamer to a target.

[00218] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

EXAMPLES

EXAMPLE 1 : APTAMER SELECTION AND SEQUENCES

EXAMPLE 1A: Selection of rRfY vWF domain A1 aptamers

[00219] Selections were performed to identify aptamers that bind to human or rabbit vWF A1 domain using a nucleotide pool consisting of 2'-OH purine and 2'-F pyrimidine nucleotides (rRFY). The selection strategy yielded high affinity aptamers specific for human and rabbit vWF A1 domains which had been immobilized on a hydrophobic plate.

Pool Preparation

[illegible]

Selection

[00221] For the human vWF A1 domain selection, the first ten rounds were initiated by immobilizing 24 pmoles of human vWF A1 domain (SEQ ID NO 4, Figure 4) to the surface of a Nunc Maxisorp hydrophobic plate (Nunc Cat.# 446612, Rochester, NY) for 1 hour at room temperature in 100 μ L of 1X Dulbecco's PBS (Gibco BRL Cat.# 14040-133, Carlsbad, CA). For Rounds eleven and twelve, 12 pmoles of full length human vWF (SEQ ID NO 7, accession number VWHU, available from Calbiochem Cat.# 681300, La Jolla, CA) were

immobilized to the hydrophobic plate. For the rabbit vWF selection, each round was initiated by immobilizing 24 pmoles of rabbit vWF A1 domain (SEQ ID NO 6: accession number AAB51555, Figure 3) under the same conditions as for human vWF A1 domain.

[00222] In all cases, after one hour of protein immobilization, the supernatant was removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. The protein-immobilized well was then blocked with 100 μ L blocking buffer (1X Dulbecco's PBS with 1% BSA) for 1 hour at room temperature. In Round one, 333 pmoles of pool RNA (2×10^{14} unique molecules) were incubated in 100 μ L 1X Dulbecco's PBS in the wells containing BSA-blocked immobilized protein target for 1 hour at room temperature. The supernatant was then removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. In later rounds, additional washes were added to increase the stringency of the positive selection step (see Tables 1 and 2). Starting at Round 2 and in all subsequent rounds, two negative selection steps were included before the positive selection step. First, the pool RNA was incubated for 1 hour at room temperature in an unblocked well to remove any plastic binding sequences from the pool. In the second negative selection step, the RNA was transferred to a BSA blocked well (not containing the protein target) for 1 hour at room temperature to remove any BSA binding sequences from the pool prior to the positive selection. Starting at Round 2 and in all subsequent rounds, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA were spiked into the positive selection reaction as non-specific competitors. In all cases, the pool RNA bound to the immobilized protein target was reverse transcribed directly in the selection plate with the addition of RT mix (Round 1: 100 μ L; Round 2+: 50 μ L; containing the 3'-primer according to SEQ ID NO 10 and Thermoscript RT (Invitrogen Cat. # 11146-016, Carlsbad, CA) followed by incubation at 65°C for 1 hour.

[00223] The resulting cDNA was used as a template for PCR (Round 1: 500 μ L; Round 2+: 250 μ L; containing the 5'-primer according to (SEQ ID NO 9), the 3'-primer according to (SEQ ID NO 10), and Taq polymerase (New England Biolabs Cat.# MO267L, Beverly, MA)). PCR reactions were done under the following conditions: a) denaturation step: 94°C for 2 minutes; b) cycling steps: 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute; c) final extension step: 72°C for 3 minutes. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR

product is reported in Tables 1 and 2 as the "PCR Threshold". The amplified pool template DNA was then isopropanol precipitated and half of the PCR product was used as template for the transcription of pool RNA for the next round of selection. The transcribed RNA pool was gel purified using a 10% polyacrylamide gel every third round. When not gel-purified, the transcribed RNA pool was desalted using two Centri-Spin 10 columns (Princeton Separations Cat. # CS-101, Adelphia, NJ). In all cases, an equivalent of one-tenth of the total transcription product was carried forward as the starting pool for the subsequent round of selection.

Table 1 Human vWF A1 domain selection conditions using an rRfY pool

Round	Target	Washes	PCR Threshold	Purification
1	24 pmol hA1	4 x 120uL	16	Desalt (2x)
2	24 pmol hA1	4 x 120uL	18	Desalt (2x)
3	24 pmol hA1	4 x 120uL	16	Gel purify
4	24 pmol hA1	8 x 120uL	15	Desalt (2x)
5	24 pmol hA1	8 x 120uL	15	Desalt (2x)
6	24 pmol hA1	8 x 120uL	15	Gel purify
7	24 pmol hA1	8 x 120uL	12	Desalt (2x)
8	24 pmol hA1	8 x 120uL	12	Desalt (2x)
9	24 pmol hA1	8 x 120uL	10	Gel purify
10	24 pmol hA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
11	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	20	Desalt (2x)
12	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	15	Gel purify

Table 2. Rabbit vWF A1 domain selection conditions using an rRfY pool

Round	Target	Washes	PCR Threshold	Purification
1	24 pmol rA1	4 x 120uL	16	Desalt (2x)
2	24 pmol rA1	4 x 120uL	18	Desalt (2x)
3	24 pmol rA1	4 x 120uL	16	Gel purify
4	24 pmol rA1	8 x 120uL	15	Desalt (2x)
5	24 pmol rA1	8 x 120uL	15	Desalt (2x)
6	24 pmol rA1	8 x 120uL	15	Gel purify
7	24 pmol rA1	8 x 120uL	12	Desalt (2x)
8	24 pmol rA1	8 x 120uL	12	Desalt (2x)
9	24 pmol rA1	8 x 120uL	10	Gel purify
10	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
11	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
12	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Gel purify

vWF domain A1 Binding Analysis

[00224] The selection progress was monitored using a sandwich filter binding assay. The 5'-³²P-labeled pool RNA (trace concentration) was incubated with either a no target protein control, 100 nM human vWF A1 domain (SEQ ID NO 4) or 100 nM rabbit vWF A1 domain (SEQ ID NO 6) in 1X Dulbecco's PBS containing 0.1 mg/mL tRNA, and 0.1 mg/mL salmon sperm DNA (in a final volume of 50 uL) for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell,

Keene, NH). The percentage of pool RNA bound to the nitrocellulose was calculated after Rounds 6, 9, and 12 with a three point screen (100 nM human vWF A1 domain, 100 nM rabbit vWF A1 domain, and a no-target control). Pool binding was compared to that of the naïve pool RNA (Round 0). The results of the rRfY pool binding analyses are found in Table 3.

Table 3. vWF A1 domain rRfY selection pool binding assays.

Selection	Pool Round	100nM human A1	100nM rabbit A1	No Protein
Naïve Pool	Round 0	11.2%	14.3%	10.5%
Human vWF A1	Round 6	16.0%	16.9%	13.8%
Rabbit vWF A1	Round 6	15.2%	17.9%	14.7%
Human vWF A1	Round 9	14.7%	14.3%	10.5%
Rabbit vWF A1	Round 9	13.7%	14.7%	10.1%
Human vWF A1	Round 12	31.8%	33.1%	13.2%
Rabbit vWF A1	Round 12	24.1%	17.7%	10.3%

[00225] When a significant positive ratio of binding of RNA in the presence of human or rabbit vWF A1 domain versus in the absence of protein was seen, the pools were cloned using the TOPO TA cloning kit (Invitrogen, Cat.# 45-0641, Carlsbad, CA) according to the manufacturer's instructions. Round 9 and 12 pool templates were cloned and sequenced (125 total sequences), producing 48 unique clones. All unique clones were transcribed, desalted, 5-³²P end-labeled, and assayed in a 3-point dot blot screen (no protein target control, 100 nM human vWF A1 domain (SEQ ID NO 5, Figure 3), or 100 nM rabbit vWF A1 domain (SEQ ID NO 6, Figure 3). The data is presented in the third and fourth columns of Table 4 below as the ratio of the fraction of the aptamer bound to the nitrocellulose in the presence of the target protein to the fraction of aptamer bound in the absence of the target protein.

[00226] Based on this initial screen, K_D s were determined for 12 of the best vWF dependent binding sequences using the dot blot assay and are reported in column 5 of Table 4 below. For K_D determination, aptamers transcripts were purified on 10% denaturing

polyacrylamide gels, 5' end labeled with γ -³²P ATP. An 8 point titration of human vWF A1 domain (SEQ ID NO 5) was used in the dot blot assay (1 μ M, 300 nM, 100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 0 nM), and K_D values were calculated by fitting the equation $y = \frac{(\max/(1+K/\text{protein})) + y_{\text{int}}}{1 + (K/\text{protein})}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). For all dot blot assays used to determine single clone K_D s in the Examples described herein, the target protein, *e.g.* human vWF A1 domain, is diluted with 1X Dulbecco's PBS buffer which includes 0.1 mg/mL BSA and incubated with labeled aptamer for 30 minutes at 24°C prior to filtration and quantitation.

Table 4. Human and rabbit vWF A1 domain rRfY aptamer binding activity*

(ND= not done)

Aptamer	Screen-Human/No Protein	Screen-Rabbit/No Protein	human A1 K_D (nM)
(AMX201.B1) (SEQ ID NO 11)	ND	ND	19
(AMX198.G1)SEQ ID NO 12	1.89	1.90	45
(AMX201.H3)SEQ ID NO 13	1.88	1.89	90
(AMX201.B3)SEQ ID NO 14	1.69	1.64	ND
(AMX201.G1)SEQ ID NO 15	2.14	2.20	190
(AMX198.C6)SEQ ID NO 16	3.03	4.62	249
(AMX201.B11)SEQ ID NO 17	1.55	1.52	ND
(AMX201.D10)SEQ ID NO 18	1.59	1.52	ND
(AMX198.C10)SEQ ID NO 19	1.40	3.39	555
(AMX201.H4) SEQ ID NO 20	1.79	1.86	ND
(AMX201.G9)SEQ ID NO 21	2.06	2.11	182

(AMX201.H11)SEQ ID NO 22	1.75	1.40	ND
(AMX201.C8)SEQ ID NO 23	2.47	1.50	0.2
(AMX201.H1)SEQ ID NO 24	2.61	2.46	189
(AMX198.E11)SEQ ID NO 25	1.03	2.37	1056
(AMX198.A10)SEQ ID NO 26	1.26	5.74	1860
(AMX201.D4)SEQ ID NO 27	2.23	2.46	ND
(AMX201.D3)SEQ ID NO 28	1.76	1.52	ND
(AMX201.A8)SEQ ID NO 29	1.82	1.51	ND
(AMX198.E5)SEQ ID NO 30	1.60	1.56	172

* used human vWF A1 domain SEQ ID NO 5 for aptamer screen and aptamer K_{Ds}

[00227] The nucleic acid sequences of the rRfY aptamers characterized in Table 4 above are given below. The unique sequence of each aptamer below begins at nucleotide 18, immediately following the sequence GGAGCGCACTCAGCCAC (SEQ ID NO 221), and runs until it meets the 3' fixed nucleic acid sequence TTTCGACCTCTCTGCTAGC (SEQ ID NO 222).

[00228] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and were selected under rRfY SELEX™ conditions wherein the purines (A and G) are 2'-OH (ribo) and the pyrimidines (U and C) are 2'-fluoro.

(AMX201.B1) SEQ ID NO 11

GGAGCGCACUCAGCCACAGAGCCUGAGUGUAUGAUCGCCUAGAUCUAUCGAUGCUUUUUCGACCUCUCUGCUAGC

(AMX198.G1) SEQ ID NO 12

GGAGCGCACUCAGCCACAACACUAAUGGGGAAAGUUAAGGAUUCUUGACCGGUGCGUUUCGACCUCUCUGCUAGC

(AMX201.H3) SEQ ID NO 13

GGAGCGCACUCAGCCACUAACGGUUGAUCUCAGGACUAAAUAGUCAACAAGGAUGCGUUUCGACCUCUCUGCUAGC

(AMX201.B3) SEQ ID NO 14

GGAGCGCACUCAGCCACAGAGCCUGAGUGUAUGAUCGCCGAGAUCUAUCGAUGCUUUUUCGACCUCUCUGCUAGC

(AMX201.G1) SEQ ID NO 15

GGAGCGCACUCAGCCACGCGUGGGGAAAUUUUAGCCUAAUUGGCUACUUGUGCGUUUCGACCUCUCUGCUAGC

(AMX198.C6) SEQ ID NO 16

GGAGCGCACUCAGCCACGGUGGUCAGUCAGUGAUUGAUUAAGUUCAGCUGUGGCGUUUCGACCUCUCUGCUAGC

(AMX201.B11) SEQ ID NO 17

GGAGCGCACUCAGCCACACCGAGGCGGAUAUCUACGAGAGGAAGUGCUGCUUGAAUUUCGACCUCUCUGCUAGC

(AMX201.D10) SEQ ID NO 18

GGAGCGCACUCAGCCACACUGAGGCGGAUAUCUACGAGAGGAAGUGCUGCUUGGAUUUCGACCUCUCUGCUAGC

(AMX198.C10) SEQ ID NO 19

GGAGCGCACUCAGCCACUGGUCCUAGCUAGUUGUACUAGCGACGCGUUCAGGUGGUUCGACCUCUCUGCUAGC

(AMX201.H4) SEQ ID NO 20

GGAGCGCACUCAGCCACUAACGGUUGAUCUCAGGACUAAUAGUCAACAAGGAUGCGUUUCGACCUCUCUGCUAGC

(AMX201.G9) SEQ ID NO 21

GGAGCGCACUCAGCCACUAACGGCGUAUCUCAGGACUAAAUAGUCAACAAGGAUGCGUUUCGACCUCUCUGCUAGC

(AMX201.H11) SEQ ID NO 22

GGAGCGCACUCAGCCACCCUGUCGUCUUUUGGUAGUCAGCCAAAAGCUAGUUGGUUGUUUCGACCUCUCUGCUAGC

(AMX201.C8) (ARC840) SEQ ID NO 23

GGAGCGCACU_CAGCCACCCUCGCAAG CAUUUUAAGAAUGA_CUUGUGCCCGUGGCUG UUUUCGACCUCUCUGCUAG
C

(AMX201.H1) SEQ ID NO 24

GGAGCGCACUCAGCCACUUUACGGUGAAAGUCUCUCGGGGUCCGAGUUACGGUGCGUUUCGACCUCUCUGCUAGC

(AMX198.E11) SEQ ID NO 25

GGAGCGCACUCAGCCACGGUAACAUTUUUCCGGCGAUUCUUUGAACGCCGUCGUGGUUCGACCUCUCUGCUAGC

(AMX198.A10) SEQ ID NO 26

GGAGCGCACUCAGCCACCAAGUUAUGCUGGCUUUGGUCUUUGACUGUCUGAGUGUUCGUUUCGACCUCUCUGCUAGC

(AMX201.D4) SEQ ID NO 27

GGAGCGCACUCAGCCACUGGGGCGUAUCUCGCACGAUAGUUCGUGUCAAGGAUGCGUUUUCGACCUCUCUGCUAGC

(AMX201.D3) SEQ ID NO 28

GGAGCGCACUCAGCCACGCCACGUCAAAUUAUGUCUACUUUGAUGUGCCCGUGGUUUCGACCUCUCUGCUAGC

(AMX201.A8) SEQ ID NO 29

GGAGCGCACUCAGCCACGCGUACACUGAUGUUGUAACAUGUACCCCGUGGUGUUUCGACCUCUCUGCUAGC

(AMX198.E5) SEQ ID NO 30

GGAGCGCACUCAGCCACUUCGACUUUCAUGUCUGAAGUCCUGCAGUGCGAGAGACGUUUCGACCUCUCUGCUAGC

[00229] While not wishing to be bound by any theory, based on the binding data presented in Table 4 above and the activity in cellular assays presented in Table 21 below for both the full length aptamers from this SELEX™ selection and the minimized aptamer sequences (see Example 2a below) the predicted generic secondary structure and predicted core nucleic acid sequence required for binding to the vWF target of all embodiments of the invention derived from this aptamer selection is depicted in Figure 10 as SEQ ID NO 217 (RNAstructure, Version 4.1, Mathews, D.H.; Disney, M.D.; Childs, J.L.; Schroeder, S.J.; Zuker, M.; and Turner, D.H., "Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure," 2004. *Proceedings of the National Academy of Sciences, US*, 101, 7287-7292).. ARC840 (SEQ ID NO 23) is one example of an aptamer having the sequence depicted in Figure 10 wherein the bold, underlined regions shown in the sequence listed above denote required bases.

EXAMPLE 1B: Selection of rRdY vWF domain A1 aptamers

[00230] Selections were performed to identify aptamers that bind to (1) human vWF A1 domain, (2) rabbit vWF A1 domain, or (3) human and rabbit vWF A1 domains using a nucleotide pool consisting of 2'-OH purine and deoxy-pyrimidine nucleotides (rRdY). The selection strategy yielded high affinity aptamers specific for human and rabbit vWF A1 domains which had been immobilized on a hydrophobic plate.

Pool Preparation

[00231] A DNA template with the sequence 5'-GGAGCGCACTCAGCCACNNNTTTCGACCTCTCTGCTAGC 3' (SEQ ID NO 8) was synthesized using an ABI EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The series of N's in the DNA template (SEQ ID NO 8) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers. The template was amplified with the primers 5'-TAATACGACTCACTATAGGAGCGCACTCAGCCAC-3' (SEQ ID NO 9) and 5'-GCTAGCAGAGAGGTCTGAAA-3' (SEQ ID NO 10) and then used as a template for in vitro transcription with T7 RNA polymerase (Y639F). Transcriptions were, typically, incubated at 37°C overnight, using 40 mM Tris pH 8.0, 40 mM DTT, 1 mM spermidine-HCl, 0.002 % Triton X-100, 4% (w/v) PEG-8000, 12 mM MgCl₂, 3 mM dCTP, 3 mM dTTP, 3

mM rGTP, 3 mM rATP, 0.5X inorganic pyrophosphatase, and 1X T7 polymerase (Y639F), and approximately .5 μ M template DNA.

Selection

[00232] For the human vWF selection, the first ten rounds were initiated by immobilizing 24 pmoles of human vWF A1 domain (SEQ ID NO 4) to the surface of a Nunc Maxisorp hydrophobic plate (Nunc, Cat.# 446612 Rochester, NY) for 1 hour at room temperature in 100 μ L of 1X Dulbecco's PBS (Gibco BRL Cat.# 14040-133, Carlsbad, CA). For Rounds eleven and twelve, 12 pmoles of full length human vWF (SEQ ID NO 7, Figure 4) were immobilized to the hydrophobic plate. For the rabbit vWF selection, each round was initiated by immobilizing 24 pmoles of rabbit vWF A1 domain (SEQ ID NO 6) under the same conditions. For the first two rounds of the human/rabbit alternating selection 12 pmoles of human vWF A1 domain (SEQ ID NO 4) and 12 pmoles of rabbit vWF A1 domain (SEQ ID NO 6) were immobilized to a hydrophobic plate as previously described. In the subsequent rounds of the alternating selection, the protein target was alternated each round between the human and rabbit vWF A1 domain (SEQ ID NOS 4 and 5, respectively), except in round 11, human full length vWF (SEQ ID NO 7) was used.

[00233] In all cases, after one hour of protein immobilization, the supernatant was removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. The protein-immobilized well was then blocked with 100 μ L blocking buffer (1X Dulbecco's PBS with 1% BSA) for 1 hour at room temperature. In Round one, 333 pmoles of pool RNA (2×10^{14} unique molecules) were incubated in 100 μ L 1X Dulbecco's PBS in the wells containing BSA-blocked immobilized protein target for 1 hour at room temperature. The supernatant was then removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. In later rounds, additional washes were added to increase the stringency of the positive selection step (see Tables 5, 6, and 7). Starting at Round 2 and in all subsequent rounds, two negative selection steps were included before the positive selection step. First, the pool RNA was incubated for 1 hour at room temperature in an unblocked well to remove any plastic binding sequences from the pool. In the second negative selection step, the RNA was transferred to a BSA blocked well (not containing the protein target) for 1 hour at room temperature to remove any BSA binding sequences from the pool prior to the positive selection. Starting at

Round 2 and in all subsequent rounds, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA were spiked into the positive selection reaction as non-specific competitors.

[00234] In all cases, the pool RNA bound to the immobilized protein target was reverse transcribed directly in the selection plate with the addition of RT mix (Round 1: 100 uL; Round 2+: 50 uL; containing the 3'-primer according to (SEQ ID NO 10) and Thermoscript RT (Invitrogen, Cat.# 11146-016, Carlsbad, CA) followed by incubation at 65°C for 1 hour. The resulting cDNA was used as a template for PCR (Round 1: 500 uL; Round 2+: 250 uL; containing the 5'-primer according to (SEQ ID NO 9), the 3'-primer according to (SEQ ID NO 10), and Taq polymerase (New England Biolabs, Cat.# MO267L, Beverly, MA)). PCR reactions were done under the following conditions: a) denaturation step: 94°C for 2 minutes; b) cycling steps: 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute; c) final extension step: 72°C for 3 minutes. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR product is reported in Tables 5, 6 and 7 as the "PCR Threshold". The amplified pool template DNA was then isopropanol precipitated and half of the PCR product was used as template for the transcription of pool RNA for the next round of selection. The transcribed RNA pool was gel purified using a 10% polyacrylamide gel every two rounds. When not gel-purified, the transcribed pool was desalted using two Centri-Spin 10 columns (Princeton Separations Cat. # CS-101, Adelphia, NJ). In all cases, an equivalent of one-tenth of the total transcription product was carried forward as the starting pool for the subsequent round of selection.

Table 5. Human vWF A1 domain selection conditions using an rRdY pool

Round	Target	Washes	PCR Threshold	Purification
1	24 pmol hA1	4 x 120uL	13	Desalt (2x)
2	24 pmol hA1	4 x 120uL	18	Desalt (2x)
3	24 pmol hA1	4 x 120uL	16	Gel purify
4	24 pmol hA1	8 x 120uL	15	Desalt (2x)
5	24 pmol hA1	8 x 120uL	15	Desalt (2x)

6	24 pmol hA1	8 x 120uL	15	Gel purify
7	24 pmol hA1	8 x 120uL	12	Desalt (2x)
8	24 pmol hA1	8 x 120uL	12	Desalt (2x)
9	24 pmol hA1	8 x 120uL	10	Gel purify
10	24 pmol hA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
11	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	20	Desalt (2x)
12	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	20	Gel purify

Table 6. Rabbit vWF A1 domain selection conditions using an rRdY pool

Round	Target	Washes	PCR Threshold	Purification
1	24 pmol rA1	4 x 120uL	13	Desalt (2x)
2	24 pmol rA1	4 x 120uL	18	Desalt (2x)
3	24 pmol rA1	4 x 120uL	10	Gel purify
4	24 pmol rA1	8 x 120uL	15	Desalt (2x)
5	24 pmol rA1	8 x 120uL	15	Desalt (2x)
6	24 pmol rA1	8 x 120uL	15	Gel purify
7	24 pmol rA1	8 x 120uL	15	Desalt (2x)
8	24 pmol rA1	8 x 120uL	12	Desalt (2x)
9	24 pmol rA1	8 x 120uL	10	Gel purify
10	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
11	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
12	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Gel purify

Table 7. Human/rabbit vWF A1 domain alternating selection conditions using an rRdY pool

Round	Target	Washes	PCR Threshold	Purification
1	12 pmol hA1/ 12pmol rA1	4 x 120uL	13	Desalt (2x)
2	12 pmol hA1/ 12pmol rA1	4 x 120uL	18	Desalt (2x)
3	24 pmol hA1	4 x 120uL	16	Gel purify
4	24 pmol rA1	8 x 120uL	15	Desalt (2x)
5	24 pmol hA1	8 x 120uL	15	Desalt (2x)
6	24 pmol rA1	8 x 120uL	15	Gel purify
7	24 pmol hA1	8 x 120uL	12	Desalt (2x)
8	24 pmol rA1	8 x 120uL	12	Desalt (2x)
9	24 pmol hA1	8 x 120uL	10	Gel purify
10	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Desalt (2x)
11	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	20	Desalt (2x)
12	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Gel purify

vWF Binding Analysis

[00235] The selection progress was monitored using a sandwich filter binding assay. The 5'-³²P-labeled pool RNA (trace concentration) was incubated with either a no target protein control, 100 nM human vWF A1 domain or 100 nM rabbit vWF A1 domain, in 1X Dulbecco's PBS containing 0.1 mg/mL tRNA, and 0.1 mg/mL salmon sperm DNA (in a final volume of 50 uL) for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell, Keene, NH). The

percentage of pool RNA bound to the nitrocellulose was calculated after Rounds 6, 9, and 12 with a three point screen (100 nM human vWF A1 domain, 100 nM rabbit vWF A1 domain, and a no-target control). Pool binding was compared to that of the naïve pool RNA (Round 0). The results of the rRdY pool binding analyses are found in Table 8.

Table 8. vWF A1 domain rRdY selection pool binding assays.

Selection	Pool Round	100nM hA1	100nM rA1	No Protein
Naïve Pool	Round 0	9.7%	10.4%	10.5%
Human vWF A1	Round 6	19.6%	19.7%	15.3%
Rabbit vWF A1	Round 6	14.3%	14.4%	12.3%
hA1/rA1	Round 6	19.8%	19.8%	15.9%
Human vWF A1	Round 9	23.8%	24.3%	15.6%
Rabbit vWF A1	Round 9	24.4%	24.0%	16.6%
hA1/rA1	Round 9	19.6%	19.4%	14.6%
Human vWF A1	Round 12	25.8%	23.0%	17.0%
Rabbit vWF A1	Round 12	20.7%	20.5%	13.8%
hA1/rA1	Round 12	25.2%	26.3%	16.8%

[00236] When a significant positive ratio of binding of RNA in the presence of human or rabbit vWF A1 domain (SEQ ID NOS 4 and 6, respectively) versus in the absence of protein was seen, the pools were cloned using the TOPO TA cloning kit (Invitrogen Cat.# 45-0641, Carlsbad, CA) according to the manufacturer's instructions. Round 9 and 12 pool templates were cloned and sequenced (185 total sequences), producing 78 unique clones within 3 sequence families. All unique clones were transcribed, desalted, 5-³²P end-labeled, and assayed in a 3-point dot blot screen (no protein target control, 100 nM human vWF A1 domain (SEQ ID NO 5), or 100 nM rabbit vWF A1 domain (SEQ ID NO 6). The data are presented in the third and fourth columns of Table 9 below as the ratio of the fraction of the aptamer bound to the nitrocellulose in the presence of the target protein to the fraction of aptamer bound in the absence of the target protein. Of the three sequence families, members of Family #1 and #2 and two individual, non-family aptamers, bound to both human vWF domain A1 (SEQ ID NO 5) and rabbit vWF domain A1 (SEQ ID NO 6).

[00237] Based on this initial screen, K_D 's were determined for 16 of the best vWF dependent binding sequences using the dot blot assay. For K_D determination, aptamers were purified on denaturing polyacrylamide gels and 5'-end labeled with γ - ^{32}P ATP. A 6 point protein titration of human vWF A1 domain (SEQ ID NO 5) was used in the dot blot assay (333 nM, 100 nM, 33 nM, 10 nM, 3 nM, 0 nM) in 1X DPBS plus 0.1 mg/mL BSA at room temperature for 30 minutes. K_D values were calculated by fitting the equation $y = (\max/(1+K/\text{protein})) + y_{\text{int}}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software).

[00238] Results of protein binding characterization are tabulated in the final column of Table 9 below.

Table 9. Human and rabbit vWF A1 domain rRdY aptamer binding activity*

#	Aptamer	Screen-Human/No Protein	Screen-Rabbit/No Protein	Human A1 K_D (nM)
1	(AMX203.D6) SEQ ID NO 31	1.92	1.57	523
2	(AMX205.H8) SEQ ID NO 32	2.04	2.84	788
3	(AMX205.H11) SEQ ID NO 33	2.18	2.41	144
4	(AMX205.A7) SEQ ID NO 34	1.24	1.37	ND
5	(AMX205.D11) SEQ ID NO 35	2.22	2.07	124
6	(AMX206.F9) SEQ ID NO 36	2.98	3.00	139
7	(AMX206.H9) SEQ ID NO 37	1.98	2.31	109
8	(AMX206.A10) SEQ ID NO 38	2.62	2.58	111
9	(AMX205.F9) SEQ ID NO 39	2.22	2.47	145
10	(AMX206.E7) SEQ ID NO 40	2.11	2.26	151
11	(AMX206.D7) SEQ ID NO 41	2.19	2.08	187
12	(AMX203.A6) SEQ	1.16	1.16	ND

	ID NO 42			
13	(AMX203.A1) SEQ ID NO 43	2.99	2.67	1148
14	(AMX203.G9) SEQ ID NO 44	1.65	1.35	1.3
15	(AMX205.H9) SEQ ID NO 45	2.36	3.14	178
16	(AMX206.D8) SEQ ID NO 46	2.80	3.76	370
17	(AMX203.F9) SEQ ID NO 47	1.45	1.29	ND
18	(AMX205.G9) SEQ ID NO 48	1.30	1.73	ND
19	(AMX205.F7) SEQ ID NO 49	3.13	2.37	1.5
20	(AMX205.H10) SEQ ID NO 50	1.88	2.47	397

* used human vWF A1 domain (SEQ ID NO 5) for aptamer screen and aptamer K_{Ds}

ND= not done

[00239] The nucleic acid sequences of the rRdY aptamers characterized in Table 9 above are described below. The unique sequence of each aptamer below begins at nucleotide 18, immediately following the sequence GGAGCGCACTCAGCCAC (SEQ ID NO 221), and runs until it meets the 3' fixed nucleic acid sequence TTTCGACCTCTCTGCTAGC (SEQ ID NO 222).

[00240] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and were selected under rRdY SELEX™ conditions wherein adenosine triphosphate and guanosine triphosphate are 2'-OH and cytidine triphosphate and thymidine triphosphate are deoxy.

vWF rRdY SELEX™ Family #1

[00241] The core target protein binding motifs for vWF rRdY Family #1 are shown in bold and underlined in the sequences below:

(AMX203.G9) (ARC842) SEQ ID NO 44
 GGAGCGCACT CAGCCACGGGGTGGGTAGACGGCGGGTATGTGGCTG GTGTCGAAGGGTTTCGACCTCTCTGCTAGC

(AMX203.F9) SEQ ID NO 47
 GGAGCGCACTCAGC CACTGAAGGGTAAGGACGAGGAGGGTATACAGTG TGCGCGTGATTTTCGACCTCTCTGCTAGC

(AMX203.A6) SEQ ID NO 42
 GGAGCGCACTCA GCCACCACGGGGACGGGTAGGGCGGGCGAGGTGGTGGC ATTAGCGTTTCGACCTCTCTGCTAGC

[00242] The predicted secondary structure and core nucleic acid sequences required for binding to the vWF target of some embodiments of the invention is depicted in Figure 12 as SEQ ID NO 218.

vWF rRdY SELEX™ Family #2

(AMX203.D6) SEQ ID NO 31
 GGAGCGCACTCAGCCACAGTTCTGTCGGTGATGAATTAGCGCGAGAGCTGTGGGACGTTTCGACCTCTCTGCTAGC

(AMX205.H8), SEQ ID NO 32
 GGAGCGCACTCAGCCACAAACGGACGGTGATGGATTAACGCGGGTTTATGGCAAGGTTTCGACCTCTCTGCTAGC

(AMX205.H11), SEQ ID NO 33
 GGAGCGCACTCAGCCACGGCAGCAGCGGTGATGGATTAGCGCGGTGTCGGTGGTGTCATTTTCGACCTCTCTGCTAGC

(AMX205.D11), SEQ ID NO 35
 GGAGCGCACTCAGCCACGGCAGCAGCGGTGATGAATTAGCGCGGTGTCGGTGGTGTCATTTTCGACCTCTCTGCTAGC

(AMX206.F9), SEQ ID NO 36
 GGAGCGCACTCAGCCACGGAGCGTCGGTGATGGATTAGCGCGGCTCCGTGGTACACATTTTCGACCTCTCTGCTAGC

(AMX206.H9), SEQ ID NO 37
 GGAGCGCACTCAGCCACGGAGCGTCGGTGATGGATTAGCGCGGTCCGTGGTACACCTTTTCGACCTCTCTGCTAGC

(AMX206.A10), SEQ ID NO 38
 GGAGCGCACTCAGCCACGGCATGACGGTGATGAATTAGCGCGGTGTCGGTGGTGTCATTTTCGACCTCTCTGCTAGC

(AMX205.F9), SEQ ID NO 39
 GGAGCGCACTCAGCCACGGAGCGTCGGTGATGGATTAGCGCGGCTCCGTGGTACGCCTTTTCGACCTCTCTGCTAGC

(AMX206.E7), SEQ ID NO 40
 GGAGCGCACTCAGCCACGGAGCGTCGGTGATGGATTAGCGCGGCTCCGTGGTACACCTTTTCGACCTCTCTGCTAGC

(AMX206.D7), SEQ ID NO 41
 GGAGCGCACTCAGCCACGGCAGCAGCGTGATGAATTAGCGCGGTGTCGGTGGTGTTATTTTCGACCTCTCTGCTAGC

(AMX203.A1), SEQ ID NO 43
 GGAGCGCACTCAGCCACAGTTCTGTCGGTGATGAATTAGCGCGGGAGCTGTGGGACGTTTCGACCTCTCTGCTAGC

(AMX205.H9), SEQ ID NO 45
 GGAGCGCACTCAGCCACGACGGTGATGGATTAGCGCGGTGGAGAAGATGCGCTGTTGTTTCGACCTCTCTGCTAGC

(AMX206.D8), SEQ ID NO 46
GGAGCGCACTCAGCCACGACGGTGATGGATTAGCGCGGTGGATCTTAACGTGCGAGTTTCGACCTCTCTGCTAGC

(AMX205.G9), SEQ ID NO 48
GGAGCGCACTCAGCCACAACCTGGTTGTCTGGTGATGGCATTAAACGCGGACCAAGGCATGTTTCGACCTCTCTGCTAGC

(AMX205.H10), SEQ ID NO 50
GGAGCGCACTCAGCCACTGTTGCCGACGGTGATGTATTAACGCGGGCAACGTTGGTGTTCGACCTCTCTGCTAGC

vWF rRdY SELEX™ single sequences

template was PCR amplified with the primers (5'- CTACCTACGATCTGACTAGC -3') (SEQ ID NO 52) and (5'- AGGAACTACATGAGAGTAAGC(OH) -3') (SEQ ID NO 53) under standard conditions. The PCR product was subjected to alkaline hydrolysis (333 mM NaOH, 90°C, 15 min) followed by precipitation. The strands were separated on a 10% denaturing polyacrylamide gel and the single stranded DNA pool, which migrated with a lower mobility, was excised from the gel, passively eluted, and precipitated with isopropanol.

Selection

[00247] For the human vWF selection, the first ten rounds were initiated by immobilizing 24 pmoles of human vWF A1 domain (SEQ ID NO 4) to the surface of a Nunc Maxisorp hydrophobic plate (Nunc, Cat.# 446612, Rochester, NY) for 1 hour at room temperature in 100 μ L of 1X Dulbecco's PBS (Gibco BRL, Cat.# 14040-133, Carlsbad, CA). For Rounds eleven and twelve, 12 pmoles of full length human vWF (SEQ ID NO 7) were immobilized to the hydrophobic plate. For the rabbit vWF selection, each round was initiated by immobilizing 24 pmoles of rabbit vWF A1 (SEQ ID NO 6) domain under the same conditions. For the first two rounds of the human/rabbit alternating selection, 12 pmoles of human vWF A1 domain (SEQ ID NO 4) and 12 pmoles of rabbit vWF A1 domain (SEQ ID NO 6) were immobilized to a hydrophobic plate as previously described. In the subsequent rounds of the alternating selection, the protein target was alternated each round between human and rabbit vWF A1 domain, except in Round 11 human full length vWF (SEQ ID NO 7) was used.

[00248] In all cases, after one hour of protein immobilization, the supernatant was removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. The protein-immobilized well was then blocked with 100 μ L blocking buffer (1X Dulbecco's PBS with 1% BSA) for 1 hour at room temperature. In Round one, 333 pmoles of pool DNA (2×10^{14} unique molecules) were incubated in 100 μ L 1X Dulbecco's PBS in the wells containing BSA-blocked immobilized protein target for 1 hour at room temperature. The supernatant was then removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. In later rounds, additional washes were added to increase the stringency of the positive selection step (see Tables 10, 11, and 12). Starting at Round 2 and in all subsequent rounds, two negative selection steps were included before the positive selection step. First, the pool DNA

was incubated for 1 hour at room temperature in an unblocked well to remove any plastic binding sequences from the pool. In the second negative selection step, the DNA was transferred to a BSA blocked well (not containing the protein target) for 1 hour at room temperature to remove any BSA binding sequences from the pool prior to the positive selection. Starting at Round 2 and in all subsequent rounds, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA were spiked into the positive selection reaction as non-specific competitors.

[00249] In all cases, the pool DNA bound to the immobilized protein target was eluted with 2 x 100 μ L washes with elution buffer (preheated to 90°C, 7 M Urea, 100 mM NaOAc pH 5.3, 3 mM EDTA) for five minutes. Both elutions were pooled and precipitated by the addition of ethanol, then amplified in an initial PCR reaction (100 μ L reactions including the 5'-primer according to SEQ ID NO 52, and the 3'-primer according to SEQ ID NO 53, and Taq polymerase (New England BioLabs, Cat.# M0267L, Beverly, MA). PCR reactions were done under the following conditions: a) denaturation step: 94°C for 2 minutes; b) cycling steps: 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute; c) final extension step: 72°C for 3 minutes. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR product is reported in Tables 10, 11 and 12 as the "PCR Threshold". 10 μ L of the PCR product was added to another 300 μ L of PCR mix for a prep-scale PCR reaction. The prep-scale PCR product was ethanol precipitated and was subjected to alkaline hydrolysis (333 mM NaOH, 90°C, 15 min). The strands were separated on a 10% denaturing polyacrylamide gel and the single stranded DNA pool, which migrated with a lower mobility, was excised from the gel, passively eluted, and precipitated with isopropanol. In all cases, an equivalent of half of the total single stranded DNA product was carried forward as the starting pool for the subsequent round of selection.

Table 10 Human vWF A1 domain selection conditions using a DNA pool

Round	Target	Washes	PCR Threshold	Purification
1	24 pmol hA1	4 x 120uL	10	Gel purify
2	24 pmol hA1	4 x 120uL	15	Gel purify

3	24 pmol hA1	4 x 120uL	13	Gel purify
4	24 pmol hA1	8 x 120uL	15	Gel purify
5	24 pmol hA1	8 x 120uL	15	Gel purify
6	24 pmol hA1	8 x 120uL	20	Gel purify
7	24 pmol hA1	8 x 120uL	10	Gel purify
8	24 pmol hA1	8 x 120uL	10	Gel purify
9	24 pmol hA1	8 x 120uL	10	Gel purify
10	24 pmol hA1	6 x 120uL; 2 x 120uL (15 min. each)	12	Gel purify
11	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	18	Gel purify
12	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	15	Gel purify

Table 11 Rabbit vWF A1 domain selection conditions using a DNA pool

Round	Target	Washes	PCR Threshold	Purification
1	24 pmol rA1	4 x 120uL	10	Gel purify
2	24 pmol rA1	4 x 120uL	13	Gel purify
3	24 pmol rA1	4 x 120uL	13	Gel purify
4	24 pmol rA1	8 x 120uL	15	Gel purify
5	24 pmol rA1	8 x 120uL	15	Gel purify
6	24 pmol rA1	8 x 120uL	20	Gel purify
7	24 pmol rA1	8 x 120uL	10	Gel purify

8	24 pmol rA1	8 x 120uL	10	Gel purify
9	24 pmol rA1	8 x 120uL	10	Gel purify
10	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	12	Gel purify
11	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Gel purify
12	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Gel purify

Table 12 Human/rabbit vWF A1 domain alternating selection conditions using a DNA pool

Round	Target	Washes	PCR Threshold	Purification
1	12 pmol hA1/ 12pmol rA1	4 x 120uL	12	Gel purify
2	12 pmol hA1/ 12pmol rA1	4 x 120uL	15	Gel purify
3	24 pmol hA1	4 x 120uL	10	Gel purify
4	24 pmol rA1	8 x 120uL	15	Gel purify
5	24 pmol hA1	8 x 120uL	15	Gel purify
6	24 pmol rA1	8 x 120uL	20	Gel purify
7	24 pmol hA1	8 x 120uL	12	Gel purify
8	24 pmol rA1	8 x 120uL	12	Gel purify
9	24 pmol hA1	8 x 120uL	12	Gel purify
10	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	12	Gel purify
11	12 pmol full length vWF	6 x 120uL; 2 x 120uL (15 min. each)	18	Gel purify
12	24 pmol rA1	6 x 120uL; 2 x 120uL (15 min. each)	10	Gel purify

vWF Binding Analysis

[00250] The selection progress was monitored using a sandwich filter binding assay. The 5'-³²P-labeled pool DNA (trace concentration) was incubated with either a no target protein control, 100 nM human vWF A1 domain (SEQ ID NO 4), or 100 nM rabbit vWF A1 domain (SEQ ID NO 6), in 1X Dulbecco's PBS containing 0.1 mg/mL tRNA, and 0.1 mg/mL salmon sperm DNA in a (final volume of 50 uL) for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell, Keene, NH). The percentage of pool DNA bound to the nitrocellulose was calculated after Rounds 6, 9, and 12 with a three point screen (no protein target control, 100 nM human vWF

A1 domain (SEQ ID NO 5), 100 nM rabbit vWF A1 domain (SEQ ID NO 6). Pool binding was compared to that of the naïve pool DNA (Round 0). The results of the DNA pool binding analyses are found in Table 13.

Table 13 vWF A1 domain DNA selection pool binding assays.

Selection	Pool Round	100nM hA1	100nM rA1	No Protein
Naïve Pool	Round 0	30.1%	35.4%	29.4%
Human vWF A1	Round 6	34.4%	36.4%	NA
Rabbit vWF A1	Round 6	37.9%	36.8%	35.6%
hA1/rA1	Round 6	47.9%	50.6%	49.0%
Human vWF A1	Round 9	30.4%	43.7%	19.1%
Rabbit vWF A1	Round 9	15.9%	35.0%	6.6%
hA1/rA1	Round 9	40.8%	49.5%	34.7%
Human vWF A1	Round 12	36.7%	45.9%	33.0%
Rabbit vWF A1	Round 12	23.7%	38.7%	13.4%
hA1/rA1	Round 12	21.4%	34.2%	16.4%

[00251] When a significant positive ratio of binding of DNA in the presence of human or rabbit vWF A1 domain versus in the absence of protein was seen, the pools were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, Cat.# 45-0641) according to the manufacturer's instructions. Round 9 and 12 pool templates were cloned and sequenced (243 total sequences), producing 106 unique clones within 8 sequence families, 41 of which bound to the vWF target and fell into the families described below.

[00252] All unique clones were assayed in a 3-point dot blot screen (no protein target control, 100 nM human vWF A1 domain (SEQ ID NO 5), or 100 nM rabbit vWF A1 domain (SEQ ID NO 6). The data are presented in the third and fourth columns of Table 14 below as the ratio of the fraction of the aptamer bound to the nitrocellulose in the presence of the target protein to the fraction of aptamer bound in the absence of the target protein.

[00253] Based on this initial screen, K_D 's were determined for 10 of the vWF dependent binding sequences. For K_D determination, aptamers were 5' end labeled with γ - ^{32}P ATP and a

competition dot blot assay was used with a constant protein concentration of 100 nM and an 8 point cold competitor DNA titration (333 nM, 100 nM, 33 nM, 10 nM, 3 nM, 1 nM, 33 pM, 0 pM) in 1X DPBS plus 0.1 mg/mL BSA at room temperature for 30 minutes. K_D values were calculated by fitting the equation $y = (\text{max}/(1+K/\text{protein})) + y_{\text{int}}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding characterization are tabulated in Table 14.

Table 14 Human and rabbit vWF A1 domain DNA aptamer binding activity*

#	Aptamer	Screen-Human/No Protein	Screen-Rabbit/No Protein	Human A1 K_D (nM)
1	1 (AMX199.B3) SEQ ID NO 54	1.76	1.99	30
2	(AMX199.D11) SEQ ID NO 55	4.19	3.42	30
3	(AMX200.G11) SEQ ID NO 56	1.73	1.28	ND
4	(AMX200.D11) SEQ ID NO 57	3.34	2.04	ND
5	(AMX200.D8) SEQ ID NO 58	2.86	1.61	ND
6	(AMX200.C11) SEQ ID NO 59	6.00	3.64	26
7	(AMX199.G10) SEQ ID NO 60	4.97	2.66	22
8	(AMX199.F7) SEQ ID NO 61	5.47	5.00	22
9	(AMX200.A7) SEQ ID NO 62	0.97	0.96	ND
10	(AMX200.B9) SEQ ID NO 63	2.54	2.06	ND
11	(AMX200.B1) SEQ ID NO 64	4.01	3.01	ND
12	(AMX199.C2) SEQ ID NO 65	5.09	4.61	17
13	(AMX200.B8) SEQ ID NO 66	4.13	3.13	ND
14	(AMX200.F11) SEQ	3.83	3.25	34

	ID NO 67			
15	(AMX200.D1) SEQ ID NO 68	1.26	1.06	ND
16	(AMX200.F9) SEQ ID NO 69	0.97	0.99	ND
17	(AMX199.B7) SEQ ID NO 70	4.08	3.65	29
18	(AMX200.D3) SEQ ID NO 71	2.68	2.41	36
19	(AMX199.C4) SEQ ID NO 72	3.60	2.85	34
20	(AMX200.E8) SEQ ID NO 73	1.04	1.03	ND
21	(AMX199.F10) SEQ ID NO 74	1.17	1.24	ND
22	(AMX199.F6) SEQ ID NO 75	1.37	1.30	ND
23	(AMX199.G5) SEQ ID NO 76	1.41	1.34	ND
24	(AMX199.F11) SEQ ID NO 77	1.44	1.35	ND
25	(AMX199.H7) SEQ ID NO 78	1.32	1.14	ND
26	(AMX199.A10) SEQ ID NO 79	1.25	1.29	ND
27	(AMX199.G1) SEQ ID NO 80	1.19	1.26	ND
28	(AMX199.F1) SEQ ID NO 81	1.32	1.36	ND
29	(AMX199.G4) SEQ ID NO 82	1.19	1.11	ND
30	(AMX200.A11) SEQ ID NO 83	1.49	1.19	ND
31	DL.159.83.31 (AMX200.H8) SEQ ID NO 84	1.86	1.27	ND
32	(AMX199.F8) SEQ ID NO 85	1.78	1.79	ND
33	(AMX199.B6) SEQ ID NO 86	2.01	1.91	ND

34	(AMX199.D8) SEQ ID NO 87	1.89	2.00	ND
35	(AMX200.E10) SEQ ID NO 88	1.69	1.82	ND
36	(AMX202.H10) SEQ ID NO 89	1.92	1.76	ND
37	(AMX202.B8) SEQ ID NO 90	1.66	1.41	ND
38	(AMX202.D6) SEQ ID NO 91	1.94	1.55	ND
39	(AMX202.A3) SEQ ID NO 92	2.28	2.10	ND
40	DL.159.83.98 (AMX202.A8) SEQ ID NO 93	1.27	1.27	ND
41	(AMX202.F6) SEQ ID NO 94	1.49	1.46	ND

* used human vWF A1 domain (SEQ ID NO 5) for aptamer screen and aptamer K_{DS}

ND = not done

[00254] The nucleic acid sequences of the DNA aptamers characterized in Table 14 above are described below. The unique sequence of each aptamer below begins at nucleotide 21, immediately following the sequence CTACCTACGATCTGACTAGC (SEQ ID NO 52), and runs until it meets the 3' fixed nucleic acid sequence GCTTACTCTCATGTAGTTCC (SEQ ID NO 223).

[00255] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and were selected under DNA SELEX™ wherein all of the nucleotides are deoxy.

[00256]

DNA SELEX™ 1, Family # 1

[00257] The predicted core nucleic acid binding sequence for DNA SELEX™ 1, Family #1 is shown in bold and underlined for aptamer AMX199.B3 (SEQ ID NO 54) and the consensus sequence (SEQ ID NO 95) below.

(AMX199.B3) SEQ ID NO 54

CTACCTACGATCTGACTAGCGGA ATGAGAATGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.C11) SEQ ID NO 59

CTACCTACGATCTGACTAGCGGAATGAGAGTGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.G11) SEQ ID NO 56

CTACCTACGATCTGACTAGCGGAACGAGAATGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX199.D11) SEQ ID NO 55

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.D8) SEQ ID NO 58

CTACCTACGATCTGACTAGCGGAATGAGAATGTTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.D11) SEQ ID NO 57

CTACCTACGATCTGACTAGCGGAATAAGAAATGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX199.F7) SEQ ID NO 61

CTACCTACGATCTGACTAGCGGAATGAGAGTGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.A7) SEQ ID NO 62

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGATGGATTGTTTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX202.D6) SEQ ID NO 91

CTACCTACGATCTGACTAGCGGAATGAGAAGGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.B1) SEQ ID NO 64

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGATGGATTGCCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX199.G10) SEQ ID NO 60

CTACCTACGATCTGACTAGCGGAATGAGAATGTTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.B8) SEQ ID NO 66

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGATGGATTGCACAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.B9) SEQ ID NO 63

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGATGGATTGCTCAGGTCTGCTGACTGCTTACTCTCATGTAGTTCC

(AMX202.B8) SEQ ID NO 90

CTACCTACGATCTGACTAGCGGAATGAGTATGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX202.H10) SEQ ID NO 89

CTACCTACGATCTGACTAGCGGAATGAGAAGGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.F9) SEQ ID NO 69

CTACCTACGATCTGACTAGCGGAATGAGGATGCTGATGGATTGGTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX202.A3) SEQ ID NO 92

CTACCTACGATCTGACTAGCGGAATGAGAGCGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX199.C2) SEQ ID NO 65

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGGTGGATTGCCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.F11) SEQ ID NO 67

CTACCTACGATCTGACTAGCGGAATGAGGATGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.D1) SEQ ID NO 68

CTACCTACGATCTGACTAGCGGAATGAGAGTGCTGATGGATTGCTCAGGTCTACTGGCTGCTTACTCTCATGTAGTTCC

(AMX199.C4) SEQ ID NO 72

CTACCTACGATCTGACTAGCGGAATGAGGATGCTGATGGATTGCACAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.D3) SEQ ID NO 71

CTACCTACGATCTGACTAGCGCAATGAGGATGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX199.B7) SEQ ID NO 70

CTACCTACGATCTGACTAGCGGGATGAGAGTGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

(AMX200.E8) SEQ ID NO 73

CTACCTACGATCTGACTAGCGGAATGAGGATGCTGGTGGATTGCTCAGGTCTGTTGGCTGCTTACTCTCATGTAGTTCC

[00258] The Consensus sequence for DNA SELEX™ 1, Family #1 is as follows:

SEQ ID NO 95

CTACCTACGATCTGACTAGCGGAATGAGRATGYTGRTGGATTGCHCAGGTCTRYTGRCTGCTTACTCTCATGTAGTTCC

Where Y = C or T, R = A or G and H = A, C or T

DNA SELEX™ 1 Family #2

(AMX199.F10) SEQ ID NO 74

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCCGTTCTGCTTACTCTCATGTAGTTCC

AMX199.F6) SEQ ID NO 75

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCCGCTCTGCTTACTCTCATGTAGTTCC

(AMX199.H7) SEQ ID NO 78

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCCGCTTCTGCTTACTCTCATGTAGTTCC

(AMX199.G5) SEQ ID NO 76

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCCGCCCTGCTTACTCTCATGTAGTTCC

(AMX199.F11) SEQ ID NO 77

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCTGCTCTGCTTACTCTCATGTAGTTCC

(AMX199.A10) SEQ ID NO 79

CTACCTACGATCTGACTAGCGGAACACTAGGTTGGTTAGGATTGGTGTGTTCCGTTTCTGCTTACTCTCATGTAGTTCC

(AMX199.G1) SEQ ID NO 80

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCCGCTTCTGCTTACTCTCATGTAGTTCC

(AMX199.G4) SEQ ID NO 82

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGTTGGTGTGTTCCGCTTCTGCTTACTCTCATGTAGTTCC

(AMX199.F1) SEQ ID NO 81

CTACCTACGATCTGACTAGCGAAACACTAGGTTGGTTAGGATTGGTGTGTTCCGCTATGCTTACTCTCATGTAGTTCC

The consensus sequence for DNA SELEX™ Family #2 is as follows:

SEQ ID NO 96

CTACCTACGATCTGACTAGCGRAACACTAGGTTGGTTAGGRTTGGTGTGTTYCYGYVHGCTTACTCTCATGTAGTTCC

Where Y= C or T, R = A or G and H = A, C or T

DNA SELEX™ 1 Family #3

(AMX199.B6) SEQ ID NO 86

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTTGGTACCTCCGGCTTACTCTCATGTAGTTCC

(AMX199.D8) SEQ ID NO 87

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTTGGCATCTTCGGCTTACTCTCATGTAGTTCC

(AMX199.F8) SEQ ID NO 85

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTTGGCACCTCTGGCTTACTCTCATGTAGTTCC

(AMX200.E10) SEQ ID NO 88

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTCGGCACCTTTGGCTTACTCTCATGTAGTTCC

(AMX202.F6) SEQ ID NO 94

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTCGGCACCTTCGGCTTACTCTCATGTAGTTCC

(AMX202.A8) SEQ ID NO 93

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTCGGCACCTTCGGCTTACTCTCATGTAGTTCC

DNA SELEX™ 1, Family #4

(AMX200.A11) SEQ ID NO 83

CTACCTACGATCTGACTAGCTGAGTAGTAGTAACCTTTTATTATGGTTTGGTGGGTCTGGCTTACTCTCATGTAGTTCC

(AMX200.H8) SEQ ID NO 84

CTACCTACGATCTGACTAGCTGAGTAGTCAGTAATTTTATTATGGTTTGGTGGGCCTGGCTTACTCTCATGTAGTTCC

EXAMPLE 1D: Selection #2 of DNA vWF Aptamers

[00259] A single set of DNA selections were done using full length human vWF and rabbit vWF domain A1 in a cross selection. While not wishing to be bound by any theory, our hypothesis is that such a selection should require successfully selected aptamers to bind to full length vWF, to bind to the A1 domain specifically and to cross react between human and rabbit proteins. The dominant sequence family from this second set of DNA selections binds to full length human vWF, rabbit vWF domain A1 and is functional in both the FACS and BIPA biological assays as described in Example 3 below.

[00260] Selections were performed to identify aptamers that bind to full length human vWF and rabbit vWF A1 domain, using a full length human vWF/rabbit vWF A1 domain alternating selection. This selection used a nucleotide pool consisting of deoxy-nucleotides (DNA). The selection strategy yielded high affinity aptamers specific for full length human vWF and rabbit vWF A1 domain which had been immobilized on a hydrophobic plate.

Pool Preparation

[00261] A DNA template with the sequence 5'-CTACCTACGATCTGACTAGCNNNGCTTACTCTCATGTAGTTCC-3' (SEQ ID NO 51) (ARC 493) was synthesized using an ABI EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The series of N's in the DNA template (SEQ ID NO 51) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers. The template was PCR amplified with the primers (5'-CTACCTACGATCTGACTAGC-3') (SEQ ID NO 52) and (5'-AGGAACTACATGAGAGTAAGC(OH)-3') (SEQ ID NO 53) under standard conditions. The PCR product was subjected to alkaline hydrolysis (333 mM NaOH, 90°C, 15 min) followed by precipitation. The strands were separated on a 10% denaturing polyacrylamide gel and the single stranded DNA pool, which migrated with a lower mobility, was excised from the gel, passively eluted, and precipitated with isopropanol.

Selection

[00262] For the first three rounds of the full length human vWF/rabbit vWF A1 domain alternating selection, 24 pmoles of full length human vWF (SEQ ID NO 7) and 24 moles of rabbit vWF A1 domain (SEQ ID NO 6) were immobilized. In the subsequent rounds, the protein target was alternated each round between full length human vWF and rabbit vWF A1 domain. In all cases, after one hour of protein immobilization, the supernatant was removed and the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. The protein-immobilized well was then blocked with 100 μ L blocking buffer (1X Dulbecco's PBS with 1% BSA) for 1 hour at room temperature. In Round one, 333 pmoles of pool DNA (2×10^{14} unique molecules) were incubated in 100 μ L 1X Dulbecco's PBS in the wells containing BSA-blocked immobilized protein target for 1 hour. The supernatant was then removed and

the wells were washed 4 times with 120 μ L 1X Dulbecco's PBS. In later rounds, additional washes were added to increase the stringency of the positive selection step (see Table 15). At Round 8, the selection was split to include a high salt wash condition as a possible means to increase the stringency of the SELEX[™] (using 1X Dulbecco's PBS + 400 mM NaCl) (see Table 15). Starting at Round 2 and in all subsequent rounds, two negative selection steps were included before the positive selection step. First, the pool DNA was incubated for 1 hour at room temperature in an unblocked well to remove any plastic binding sequences from the pool. In the second negative selection step, the DNA was transferred to a BSA blocked well (not containing the protein target) for 1 hour at room temperature to remove any BSA binding sequences from the pool prior to the positive selection. Starting at Round 2 and in all subsequent rounds, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA were spiked into the positive selection reaction as non-specific competitors.

[00263] In all cases, the pool DNA bound to the immobilized protein target was eluted with 2 x 100 μ L washes with elution buffer (preheated to 90°C, 7 M Urea, 100 mM NaOAc pH 5.3, 3 mM EDTA) for five minutes. Both elutions were pooled and precipitated by the addition of ethanol, then amplified in an initial PCR reaction (100 μ L reactions including the 5'-primer according to SEQ ID NO 52, the 3'-primer according to SEQ ID NO 53, and Taq polymerase, (New England BioLabs, Cat.# M0267L, Beverly, MA). PCR reactions were done under the following conditions: a) denaturation step: 94°C for 2 minutes; b) cycling steps: 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute; c) final extension step: 72°C for 3 minutes. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR product is reported in Table 15 as the "PCR Threshold". 10 μ L of the PCR product was added to another 300 μ L of PCR mix for a prep-scale PCR reaction. The prep-scale PCR product was ethanol precipitated and was subjected to alkaline hydrolysis (333 mM NaOH, 90°C, 15 min). The strands were separated on a 10 % denaturing polyacrylamide gel and the single stranded DNA pool, which migrated with a lower mobility, was excised from the gel, passively eluted, and precipitated with isopropanol. In all cases, an equivalent of half of the total single stranded DNA product was carried forward as the starting pool for the subsequent round of selection.

Table 15 Full length human vWF/rabbit vWF A1 domain alternating selection conditions using a DNA pool

Round	Target	Washes		PCR Threshold		Purification
1	24 pmol full length human / 24 pmol rA1	4 x 120uL		15		Gel purify
2	24 pmol full length human / 24 pmol rA1	4 x 120uL		13		Gel purify
3	24 pmol full length human / 24 pmol rA1	4 x 120uL		10		Gel purify
4	24 pmol full length human vWF	4 x 120uL		10		Gel purify
5	24 pmol rA1	4 x 120uL		10		Gel purify
6	24 pmol full length human vWF	8 x 120uL		10		Gel purify
7	24 pmol rA1	8 x 120uL		10		Gel purify
		Normal Wash	High Salt Wash	Normal Wash	High Salt Wash	
8	24 pmol full length human vWF	8 x 120uL	8 x 120uL	10	10	Gel purify
9	24 pmol rA1	8 x 120uL	8 x 120uL	10	13	Gel purify
10	24 pmol full length human vWF	8 x 120uL	8 x 120uL	10	10	Gel purify
11	24 pmol rA1	8 x 120uL	8 x 120uL	10	10	Gel purify

vWF Binding Analysis

[00264] The selection progress was monitored using a sandwich filter binding assay. The 5'-³²P-labeled pool DNA (trace concentration) was incubated with either a no target protein control, 100 nM full length human vWF (Calbiochem Cat.# 681300, La Jolla, CA), or 100 nM rabbit vWF A1 domain, in 1X Dulbecco's PBS containing 0.1 mg/mL tRNA, and 0.1 mg/mL salmon sperm DNA, and 0.1 mg/mL BSA in a (final volume of 50 uL) for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell, Keene, NH). The percentage of pool DNA bound to the

nitrocellulose was calculated after Rounds 7 and 9 by screening with a no protein target control, 30 nM /100 nM full length human vWF (Calbiochem Cat.# 681300, La Jolla, CA), and 30 nM/100 nM rabbit vWF A1 domain (SEQ ID NO 6). Pool binding was compared to that of the naïve pool DNA (Round 0). The results of the DNA pool binding analyses are found in Table 16 below.

Table 16 full length human vWF/ rabbit vWF A1 domain DNA selection pool binding assays.

Selection	Pool Round	full length human vWF		rabbit vWF A1 domain		No Protein
		30nM	100nM	30nM	100nM	
Naïve Pool	Round 0	40.3%	39.8%	41.6%	45.5%	35.2%
Human vWF/rA1	Round 7	59.4%	66.9%	58.9%	68.3%	39.9%
Naïve Pool	Round 0	53.0%	55.1%	53.9%	56.9%	52.6%
Human vWF/rA1	Round 9	70.9%	65.0%	71.7%	81.6%	54.5%
Human vWF/rA1 High Salt Wash	Round 9	72.1%	73.8%	74.5%	82.3%	59.7%

[00265] When a significant positive ratio of binding of DNA in the presence of human or rabbit vWF A1 domain versus in the absence of protein was seen, the pools were cloned using the TOPO TA cloning kit (Invitrogen Cat.# 45-0641, Carlsbad, CA) according to the manufacturer's instructions. Round 7 and 11 pool templates were cloned and sequenced (218 total sequences), producing 146 unique clones within 12 sequence families of which sequences from six families show vWF target binding activity. All unique clones were assayed twice in a 3-point dot blot screen (no protein target control, 20 nM full length human vWF (Calbiochem Cat.# 681300, La Jolla, CA), or 20 nM rabbit vWF A1 domain. The data is presented in the third and fourth columns of Table 17 below as the ratio of the fraction of

the aptamer bound to the nitrocellulose in the presence of the target protein to the fraction of aptamer bound in the absence of the target protein.

[00266] Based on this initial screen, K_D s were determined for 3 of the vWF dependent binding sequences using the dot blot assay. For K_D determination, aptamers were 5' end labeled with γ - 32 P ATP and were tested for direct binding to full length human vWF and rabbit vWF A1 domain. A 12 point protein titration was used in the dot blot assay (300 nM, 100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 3 pM, 0 pM) in 1X DPBS plus 0.1 mg/mL BSA at room temperature for 30 minutes. K_D values were calculated by fitting the equation $y = (\text{max}/(1+K/\text{protein}))+y_{\text{int}}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding characterization are tabulated in Table 17 below.

Table17 Full length human vWF and rabbit vWF A1 domain DNA aptamer binding activity*

#	Aptamer	Screen-Human/ No Protein	Screen Rabbit/ No Protein	full length human vWF K_D (nM)	rabbit vWF A1 domain K_D (nM)
1	AMX237.A11 (SEQ ID NO 98)	2.66	2.38	ND	ND
2	AMX237.A2 (SEQ ID NO 99)	2.60	2.34	ND	ND
3	AMX238.D12 (SEQ ID NO 100)	2.40	2.19	ND	ND
4	AMX237.H5 (SEQ ID NO 101)	1.63	1.61	ND	ND
5	AMX237.E2 (SEQ ID NO 102)	1.61	1.61	ND	ND
6	AMX237.B4 (SEQ ID NO 103)	1.42	1.38	ND	ND
7	AMX237.E9	2.13	2.06	ND	ND

	(SEQ ID NO 104)				
8	AMX237.D11 (SEQ ID NO 105)	1.16	1.17	ND	ND
9	AMX238.G5 (SEQ ID NO 106)	3.06	2.68	ND	ND
10	AMX237.C7 (SEQ ID NO 107)	1.15	1.16	ND	ND
11	AMX238.D5 (SEQ ID NO 108)	1.46	1.40	ND	ND
12	AMX237.B11 (SEQ ID NO 109)	2.87	2.57	ND	ND
13	AMX237.F6 (SEQ ID NO 110)	1.20	1.21	ND	ND
14	AMX238.D8 (SEQ ID NO 111)	2.02	1.97	ND	ND
15	AMX238.G6 (SEQ ID NO 112)	1.25	1.22	ND	ND
16	AMX236.F8 (SEQ ID NO 113)	1.14	1.13	ND	ND
17	AMX237.G6 (SEQ ID NO 114)	3.80	3.63	0.20	47
18	AMX238.E9 (SEQ ID NO 115)	3.44	3.36	0.39	5.3
19	AMX238.E7 (SEQ ID NO 116)	3.02	2.83	ND	ND
20	AMX238.F3 (SEQ ID NO 117)	2.83	2.72	ND	ND
21	AMX238.H5	3.75	3.46	0.33	6.0

	(SEQ ID NO 118)				
22	AMX237.C11 (SEQ ID NO 119)	2.04	1.95	ND	ND
23	AMX238.F2 (SEQ ID NO 120)	2.84	2.76	ND	ND
24	AMX237.F9 (SEQ ID NO 121)	2.21	2.31	ND	ND
25	AMX237.F12 (SEQ ID NO 122)	1.95	2.08	ND	ND
26	AMX237.C9 (SEQ ID NO 123)	2.05	2.19	ND	ND
27	AMX237.F10 (SEQ ID NO 124)	2.90	2.90	ND	ND
28	AMX236.H2 (SEQ ID NO 125)	2.12	2.06	ND	ND
29	AMX237.C5 (SEQ ID NO 126)	2.55	2.36	ND	ND
30	AMX236.A12 (SEQ ID NO 127)	2.64	2.41	ND	ND
31	AMX236.B8 (SEQ ID NO 128)	1.66	1.88	ND	ND
32	AMX236.A11 (SEQ ID NO 129)	2.02	2.02	ND	ND
33	AMX237.D5 (SEQ ID NO 130)	1.41	1.48	ND	ND
34	AMX236.E6 (SEQ ID NO	1.31	1.49	ND	ND

	131)				
35	AMX236.C12 (SEQ ID NO 132)	1.99	2.24	ND	ND
36	AMX237.H10 (SEQ ID NO 133)	1.71	1.94	ND	ND
37	AMX237.G7 (SEQ ID NO 134)	2.68	2.54	ND	ND
38	AMX237.H8 (SEQ ID NO 135)	1.21	1.41	ND	ND
39	AMX236.G4 (SEQ ID NO 136)	1.70	1.72	ND	ND
40	AMX236.C1 (SEQ ID NO 137)	1.03	3.28	ND	ND
41	AMX237.E10 (SEQ ID NO 138)	1.12	6.04	ND	ND
42	AMX238.F5 (SEQ ID NO 139)	1.05	4.40	ND	ND
43	AMX237.C1 (SEQ ID NO 140)	0.76	3.47	ND	ND
44	AMX237.B12 (SEQ ID NO 141)	1.13	4.67	ND	ND
45	AMX238.A6 (SEQ ID NO 142)	0.92	3.47	ND	ND
46	AMX238.A11 (SEQ ID NO 143)	0.85	4.54	ND	ND
47	AMX236.C6 (SEQ ID NO 144)	1.06	5.77	ND	ND

48	AMX238.F6 (SEQ ID NO 145)	1.18	5.36	ND	ND
49	AMX236.E2 (SEQ ID NO 146)	0.93	3.59	ND	ND
50	AMX238.G4 (SEQ ID NO 147)	1.09	1.39	ND	ND
51	AMX238.H9 (SEQ ID NO 148)	1.11	1.32	ND	ND
52	AMX237.B1 (SEQ ID NO 149)	2.00	2.10	ND	ND
53	AMX238.A3 (SEQ ID NO 150)	1.36	1.03	ND	ND
54	AMX237.C4 (SEQ ID NO 151)	0.97	1.31	ND	ND
55	AMX237.E5 (SEQ ID NO 152)	0.97	1.15	ND	ND
56	AMX237.F1 (SEQ ID NO 153)	0.98	1.22	ND	ND
57	AMX237.F5 (SEQ ID NO 154)	0.99	1.22	ND	ND
58	AMX238.H11 (SEQ ID NO 155)	0.98	1.14	ND	ND
59	AMX237.G2 (SEQ ID NO 156)	1.02	1.16	ND	ND
60	AMX238.A12 (SEQ ID NO 157)	1.23	0.99	ND	ND
61	AMX236.C9	1.24	1.00	ND	ND

	(SEQ ID NO 158)				
62	AMX236.H1 (SEQ ID NO 159)	1.10	1.14	ND	ND
63	AMX236.F7 (SEQ ID NO 160)	1.18	1.20	ND	ND
64	AMX236.B3 (SEQ ID NO 161)	1.54	1.41	ND	ND
65	AMX238.D9 (SEQ ID NO 162)	1.22	0.97	ND	ND
66	AMX238.F7 (SEQ ID NO 163)	1.20	1.88	ND	ND
67	AMX236.G1 (SEQ ID NO 164)	1.47	1.51	ND	ND

* used full length human vWF (SEQ ID NO 7) and rabbit vWF A1 domain (SEQ ID NO 6) for aptamer screen and aptamer K_D s

ND= not done

[00267] The nucleic acid sequences of the DNA aptamers characterized in Table 17 above are described below. The unique sequence of each aptamer below begins at nucleotide 21, immediately following the sequence CTACCTACGATCTGACTAGC (SEQ ID NO 52), and runs until it meets the 3' fixed nucleic acid sequence GCTTACTCTCATGTAGTTCC (SEQ ID NO 223).

[00268] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and were selected under DNA SELEX™ wherein all of the nucleotides are deoxy.

vWF DNA SELEX™ 2, Family 1.1

[00269] Families 1.1 and 1.2 yielded the parent of ARC1029 (SEQ ID NO 214). The predicted core nucleic acid binding sequences to the target von Willebrand Factor are

underlined and shown in bold for aptamers AMX237.E9 (SEQ ID NO 104) and AMX238.H5 (SEQ ID NO 118) below.

AMX237.E9 (SEQ ID NO 104)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCTAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX237.B11 (SEQ ID NO 109)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCTAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX237.A11 (SEQ ID NO 98)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX238.G5 (SEQ ID NO 106)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX238.D8 (SEQ ID NO 111)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAACAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX237.E2 (SEQ ID NO 102)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCTAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX237.H5 (SEQ ID NO 101)

CTACCTACGATCTGACTAGCTCCAGTGTTCATTTAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX238.D5 (SEQ ID NO 108)

CTACCTACGATCTGAGTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGTCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX237.A2 (SEQ ID NO 99)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX238.D12 (SEQ ID NO 100)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCCTCCGTGAGCTTACTCTCATGTAGTTCC

AMX237.F6 (SEQ ID NO 110)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCTAATAACCGTGCGGTGCCTCCGTGATGCTTACTCTCATGTAGTTCC

AMX237.D11 (SEQ ID NO 105)

CTACCTACGATCTGACTAGCTCCAGTGTTCATATAATAACCGTGCGGTGCCTCCGTGATGCTTACTCTCATGTAGTTCC

AMX237.B4 (SEQ ID NO 103)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCTTCCGTGAGCTTACTCTCATGTAGTTCC

AMX236.F8 (SEQ ID NO 113)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCCTCCGTGATGCTTACTCTCATGTAGTTCC

AMX237.C7 (SEQ ID NO 107)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGTGCCTCCGTGATGCTTACTCTCATGTAGTTCC

AMX238.G6 (SEQ ID NO 112)

CTACCTACGATCTGACTAGCTCCAGTGTTCATCCAATAACCGTGCGGGGCCTCCGTGATGCTTACTCTCATGTAGTTCC

vWF DNA SELEX™ 2, Family # 1.2

AMX238.H5 (SEQ ID NO 118)

CTACCTACGATCTGACTAGCGTGCAGTGCCTATTCTAGGCCGTGCGGTGCCTCCGTACGCCTTACTCTCATGTAGTTCC

AMX237.C11 (SEQ ID NO 119)

CTACCTACGATCTGACTAGCGTGCA GTGCCTATTCTAGGCCGTGCGGTGCCTCCGTCATGCTTACTCTCATGTAGTTCC

AMX238.E7 (SEQ ID NO 116)

CTACCTACGATCTGACTAGCGTGCA GTGCCTATTTTAGGCCGTGCGGTGCCTCCGTCACGCTTACTCTCATGTAGTTCC

AMX237.G6 (SEQ ID NO 114)

CTACCTACGATCTGACTAGCGTGCA GTGCCTATCCAGGCCGTGCGGTGCCTCCGTCACGCTTACTCTCATGTAGTTCC

AMX238.F2 (SEQ ID NO 120)

CTACCTACGATCTGACTAGCATGCAGTGCCCATTTCTAGGCCGTGCGGTGCCTCCGTCATGCTTACTCTCATGTAGTTCC

AMX238.E9 (SEQ ID NO 115)

CTACCTACGATCTGACTAGCGTGCA GTGCCCATCTTAGGCCGTGCGGTGCCTCCGTCACGCTTACTCTCATGTAGTTCC

AMX238.F3 (SEQ ID NO 117)

CTACCTACGATCTGACTAGCGTGCA GTGCCTATTTTAGGTCGTGCGGGGCCTCCGTCACGCTTACTCTCATGTAGTTCC

AMX237.F10 (SEQ ID NO 124)

CTACCTACGATCTGACTAGCGTGCA GTGCCCATCCAGGCCGTGCGGTATCCTCCGTCACGCTTACTCTCATGTAGTTCC

AMX237.C5 (SEQ ID NO 126)

CTACCTACGATCTGACTAGCGTGCA GTGCCTATCTCAGGCCGTGCGGTATCCTCCGTCACGCTTACTCTCATGTAGTTCC

AMX236.H2 (SEQ ID NO 125)

CTACCTACGATCTGACTAGCGTGCA GTGCCTATCCCAGGCCGTGCGGTAGCCTCCGTCACGCTTACTCTCATGTAGTTCC

[00270] The predicted secondary structure and core nucleic acid sequences required for binding to the vWF target of some embodiments of the invention comprised in Family#1 of this aptamer selection is depicted in Figure 15 as SEQ ID NO 220.

vWF DNA SELEX™ 2, Binding Family # 2

AMX237.C9 (SEQ ID NO 123)

CTACCTACGATCTGACTAGCTTGGTAGTGACTTTGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX237.F12 (SEQ ID NO 122)

CTACCTACGATCTGACTAGCTTGGTAGCGATTTGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX237.F9 (SEQ ID NO 121)

CTACCTACGATCTGACTAGCTTGGTAGCGATTCTGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX237.G7 (SEQ ID NO 134)

CTACCTACGATCTGACTAGCTTGGTAGCGACTTTGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX236.A12 (SEQ ID NO 127)

CTACCTACGATCTGACTAGCTTGGTAGCGACTCTGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX236.G4 (SEQ ID NO 136)

CTACCTACGATCTGACTAGCTTGGTAGCGACTTTGTGGAGATGCGGTTTGGTTGACGTCAGCTTACTCTCATGTAGTTCC

AMX236.C12 (SEQ ID NO 132)

CTACCTACGATCTGACTAGCTTGGTAGCGACTCCGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX236.A11 (SEQ ID NO 129)

CTACCTACGATCTGACTAGCTTGGTAGCGACTCTGTGGAGCTGCGGTCTGGCCGACGTCAGCTTACTCTCATGTAGTTCC

AMX236.E6 (SEQ ID NO 131)

CTACCTACGATCTGACTAGCTTGGTAGCGACCCTGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX236.B8 (SEQ ID NO 128)

CTACCTACGATCTGACTAGCTTGGTAGCGACTCTGTGGAGCTGCGGTCTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX237.H8 (SEQ ID NO 135)

CTACCTACGATCTGACTAGCTTGGTAGCGACTTGTGGAGCTGCGGTTTGGTCGACATCAGCTTACTCTCATGTAGTTCC

AMX237.D5 (SEQ ID NO 130)

CTACCTACGATCTGACTAGCTTGGTAGCGACTGTGGAGCTGCGGTTTGGTTGACGTCAGCTTACTCTCATGTAGTTCC

AMX237.H10 (SEQ ID NO 133)

CTACCTACGATCTGACTAGCTTGGTAGCGACTCAGAGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

AMX237.B1 (SEQ ID NO 149)

CTACCTACGATCTGACTAGCTTGGTAGCGACACAGTGGAGCTGCGGTTTGGTCGACGTCAGCTTACTCTCATGTAGTTCC

The consensus sequence for DNA SELEX™ 2 Family #2 is as follows:

SEQ ID NO 325

CTACCTACGATCTGACTAGCTTGGTAG Y GA Y (Y/A) Y (Y/A) G (T/A) GGAG (C/A) TGC GGT Y TGG YY GAC R
TCAGCTTACTCTCATGTAGTTCC

Where Y= C or T, R = A or G and (Y/A)=C, T or A,

vWF DNA SELEX™ 2, Binding Family # 3

[00271] This family is equivalent to vWF DNA SELEX™ 1, Family # 1 described above in that the sequences in both families are more than 90% identical.

AMX238.A11 (SEQ ID NO 143)

CTACCTACGATCTGACTAGCGGAATGAGAGTGTTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX237.C1 (SEQ ID NO 140)

CTACCTACGATCTGACTAGCGGAATGAGGATGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX236.C6 (SEQ ID NO 144)

CTACCTACGATCTGACTAGCGGAATGAGAGTGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX236.C1 (SEQ ID NO 137)

CTACCTACGATCTGACTAGCGGAATGAGAATGTTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX237.E10 (SEQ ID NO 138)

CTACCTACGATCTGACTAGCGGAATGAGAATGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX238.F6 (SEQ ID NO 145)

CTACCTACGATCTGACTAGCGGAATGAGTATGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX236.E2 (SEQ ID NO 146)

CTACCTACGATCTGACTAGCGGAATGAGTATGCTGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX237.B12 (SEQ ID NO 141)

CTACCTACGATCTGACTAGCGGAATGAGAATGCAGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX238.A6 (SEQ ID NO 142)

CTACCTACGATCTGACTAGCGGAATGAGAATGCAGATGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

AMX238.F5 (SEQ ID NO 139)

CTACCTACGATCTGACTAGCGGAATGAGAAGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCATGTAGTTCC

vWF DNA SELEXTM 2, Binding Family# 4

AMX237.G2 (SEQ ID NO 156)

CTACCTACGATCTGACTAGCTTTTCAGTCTTTCATATTTATAGGGTTTGGCATTGGGTCTGGCTTACTCTCATGTAGTTCC

AMX237.C4 (SEQ ID NO 151)

CTACCTACGATCTGACTAGCTTTTCAGTCTTCCACATTTATAGGGTTTGGCATTGGGTCTGGCTTACTCTCATGTAGTTCC

AMX237.F5 (SEQ ID NO 154)

CTACCTACGATCTGACTAGCTTTTAGTCTTCCACATTTATAGGGTTTGGCATTGGGTCTGGCTTACTCTCATGTAGTTCC

AMX238.H11 (SEQ ID NO 155)

CTACCTACGATCTGACTAGCTTTTCAGTCTTTCACATTTATAGGGTTTGGCATTGGGTCTGGCTTACTCTCATGTAGTTCC

AMX238.G4 (SEQ ID NO 147)

CTACCTACGATCTGACTAGCTTGTGCGCACTTTGGTTGGTCTGGTTGGTTCTAAGTGGCGCTTACTCTCATGTAGTTCC

AMX237.E5 (SEQ ID NO 152)

CTACCTACGATCTGACTAGCTTTTCAGTCTTCTACATTTATAGGGTTTGGCATTGGGTCTGGCTTACTCTCATGTAGTTCC

AMX238.H9 (SEQ ID NO 148)

CTACCTACGATCTGACTAGCTTGTGCGCACTTTGGTTGGTCTGGTTGGTTTAAAGTGGCGCTTACTCTCATGTAGTTCC

AMX237.F1 (SEQ ID NO 153)

CTACCTACGATCTGACTAGCTTTTCAGTCTTCCACGTTTATAGGGTTTGGCATTGGGTCTGGCTTACTCTCATGTAGTTCC

vWF DNA SELEXTM 2, Family #5

AMX236.G1 (SEQ ID NO 164)

CTACCTACGATCTGACTAGCCTCAGATTGACTCCGGCTGACTTGTTTTAATCTTCTGAGTGCTTACTCTCATGTAGTTCC

AMX236.B3 (SEQ ID NO 161)

CTACCTACGATCTGACTAGCCTTACCTATCCCTTCTGCGGAATACGTCGAGTACTATGCTTACTCTCATGTAGTTCC

AMX236.F7 (SEQ ID NO 160)

CTACCTACGATCTGACTAGCCCCACTTATCGTGACCTTATGATATGTCGAATACTCTTGCTTACTCTCATGTAGTTCC

AMX236.H1 (SEQ ID NO 159)

CTACCTACGATCTGACTAGCCTCAGATTGACTCCGGCCGACTTGTTTTAATCTTCTGAGTGCTTACTCTCATGTAGTTCC

The consensus sequences for DNA SELEX™ 2 Family #5 are as follows:

SEQ ID NO 326

Family5.1 = SEQ ID NO 164 and SEQ ID NO 159

CTACCTACGATCTGACTAGCCTCAGATTGACTCCGGCYGACTTGTTTAACTCTTGAGTGCTTACTCTCATGTAGTTCC

Wherein Y = Cor T
SEQ ID NO 327

Family5.2 = SEQ ID NO 161 & SEQ ID NO 160

CTACCTACGATCTGACTAGCC YY AC Y TAT Y (C/G) Y (C/G) T (T/A) C Y (G/T) Y R (G/T) R ATA Y GTCGA R TACT (A/C)
TGCTTACTCTCATGTAGTTCC

Where Y= C or T, R = A or G

vWF DNA SELEX™ 2, Family # 6

AMX238.A3 (SEQ ID NO 150)

CTACCTACGATCTGACTAGCTCAAAGTATTACTTATTGGCAATAAGTCGTTTACTCTATAGCTTACTCTCATGTAGTTCC

AMX238.F7 (SEQ ID NO 163)

CTACCTACGATCTGACTAGCAAGGGGATTGGCTCCGGGTCTGGCGTGCTTGGCATCTTTGGCTTACTCTCATGTAGTTCC

AMX236.C9 (SEQ ID NO 158)

CTACCTACGATCTGACTAGCCAGTTCTGGGAAAAATTATTTTTTATTTTCGATCGTATTTGCTTACTCTCATGTAGTTCC

AMX238.D9 (SEQ ID NO 162)

CTACCTACGATCTGACTAGCCAGTTCTGGGAAAAATCATTTTTTATTTTCGATCGTATTTGCTTACTCTCATGTAGTTCC

AMX238.A12 (SEQ ID NO 157)

CTACCTACGATCTGACTAGCCAGTTCTGGGAAAAATTATTTTTTATTTTCGATCGTATATGCTTACTCTCATGTAGTTCC

EXAMPLE 2: COMPOSITION AND SEQUENCE OPTIMIZATION AND SEQUENCES

EXAMPLE 2A: Truncation of rRfY vWF aptamers

[00272] On the basis of the vWF binding analysis described in Example 1 above and the cell based assay data described in Example 3 below, aptamer ARC840 (AMX201.C8) (SEQ ID NO 23) was chosen from the rRfY selections for further characterization.

[00273] In order to identify the core structural elements required for vWF binding, the 3'-boundary of ARC840 (AMX201.C8) (SEQ ID NO 23) was determined. The full length RNA transcript was labeled at the 5'-end with γ -³²P ATP and T4 polynucleotide kinase. Radiolabeled ligands were subjected to partial alkaline hydrolysis and then selectively bound in solution to human von Willebrand Factor A1 domain (SEQ ID NO 5) at 500 nM before being passed through nitrocellulose filters. Both the retained and the not retained oligonucleotides were resolved separately on 8 % denaturing polyacrylamide gels. The smallest oligonucleotide bound to vWF defined the 3'- boundary. On the basis of the boundary experiments as well as visual inspection of predicted folds, a panel of minimized sequences was designed. Folds of all the nucleic acid sequences of the invention were predicted using RNAstructure, Version 4.1 downloaded from the University of Rochester. RNAstructure is a Windows implementation of the Zuker algorithm for RNA secondary structure prediction based on free energy minimization (Mathews, D.H.; Disney, M.D.; Childs, J.L.; Schroeder, S.J.; Zuker, M.; and Turner, D.H., "Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure," 2004. *Proceedings of the National Academy of Sciences, US*, 101, 7287-7292). RNAstructure 4.1 uses the most current thermodynamic parameters from the Turner lab.

[00274] For the minimized rRfY aptamers, described below, the purines comprise a 2'-OH and the pyrimidines comprise a 2'-F modification, while, the templates and primers comprise unmodified deoxyribonucleotides.

[00275] For the minimized rRfY aptamer 5'-
GGAGCGCACUCAGCCACCCUCGCAAGCAUUUUAAGAAUGACUUGUGCCGCUGG
CUG-3' (SEQ ID NO 165), the 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA
-3' (SEQ ID NO 166) and 3' PCR primer 5'- CAGCCAGCGGCACAAGTC -3' (SEQ ID
NO 167) were used to amplify template 5'-
TCGATCTAATACGACTCACTATAGGAGCGCACTCAGCCACCCTCGCAAGCATTTT
AAGAATGACTTGTGCCGCTGGCTG-3' (SEQ ID NO 168).

[00276] For minimized aptamer 5'-
GGACCACCCUCGCAAGCAUUUUAAGAAUGACUUGUGCCGCUGGUCC -3'(SEQ ID

NO 169), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'- GGACCAGCGGCACAAGTC -3' (SEQ ID NO 170) were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGACCACCCTCGCAAGCATTTTAAGAATGACCTTGCGCGCTGGTCC -3'(SEQ ID NO171).

[00277] For minimized aptamer 5'-

GGACCACCCUCGCAAGCAUUGAGAAAUGACUUGUGCCGCUGGUCC -3' (SEQ ID NO 172), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'- GGACCAGCGGCACAAGTC -3' (SEQ ID NO 170) were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGACCACCCTCGCAAGCATTTGAGAAATGACCTTGCGCGCTGGTCC -3'(SEQ ID NO 173).

[00278] For minimized aptamer 5'-

GGACCACCCUCGCAACGAGAGUUGUGCCGCUGGUCC -3' (SEQ ID NO 174), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO166) and 3' PCR primer 5'- GGACCAGCGGCACAAGTC -3' (SEQ ID NO 175) were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGACCACCCTCGCAACGAGAGTTGTGCGCGCTGGTCC -3' (SEQ ID NO 176).

[00279] All of the above minimized aptamer sequences were transcribed, gel-purified on 15% denaturing polyacrylamide gels, 5-³²P end-labeled with γ^{32} P ATP, and then desalted using two Centri-Spin 10 columns (Princeton Separations Cat.# CS-101, Adelphia, NJ). These minimers were primarily characterized in the cellular assays described in Example 3 below.

EXAMPLE 2B: Truncation of rRdY vWF Aptamers

[00280] On the basis of the vWF binding analysis described in Example 1 above and cell based assay data described in Example 3 below, aptamers AMX203.G9 (SEQ ID NO 44) and AMX205.F7 (SEQ ID NO 49), respectively, were identified for further characterization.

[00281] In order to identify the core structural elements required for vWF binding, the 3'-boundaries of aptamers AMX203.G9 (SEQ ID NO 44) and AMX205.F7 (SEQ ID NO 49) were determined. The full length RNA transcripts were labeled at the 5'-end with γ -³²P ATP and T4 polynucleotide kinase. Radiolabeled ligands were subjected to partial alkaline hydrolysis and then selectively bound in solution to human vWF A1 domain (SEQ ID NO 5) at 500 nM before being passed through nitrocellulose filters. Retained oligonucleotides were resolved on 8% denaturing polyacrylamide gels. The smallest oligonucleotide bound to vWF defined the 3'-boundary. On the basis of the boundary experiments as well as visual inspection of predicted folds using RNAstructure, Version 4.1, a panel of minimized sequences was designed.

[00282] For the minimized rRdY aptamers, described below, the purines are 2'-OH purines and the pyrimidines are deoxy-pyrimidines, while the templates and primers comprise unmodified deoxyribonucleotides. The following three minimized aptamer sequences were derived from DL.159.87.70 (SEQ ID NO 44):

[00283] For minimized aptamer sequence 5'-GGAGCGCACTCAGCCACGGGGTGGGTAGACGGCGGGTATGTGGCT -3' (SEQ ID NO 177), 5' PCR primer 5'-GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'-AGCCACATACCCGCCGTC -3' (SEQ ID NO 178) were used to amplify template 5'-GATCGATCTAATACGACTCACTATAGGAGCGCACTCAGCCACGGGGTGGGTAGACGGCGGGTATGTGGCT -3' (SEQ ID NO 179).

[00284] For minimized aptamer sequence 5'-GGAGCCACGGGGTGGGTAGACGGCGGGTATGTGGCTCC -3' (SEQ ID NO 180), 5' PCR primer 5'-GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'-GGAGCCACATACCCGCCG -3' (SEQ ID NO 181), were used to amplify template 5'-GATCGATCTAATACGACTCACTATAGGAGCCACGGGGTGGGTAGACGGCGGGTATGTGGCTCC -3' (SEQ ID NO 182).

[00285] For minimized aptamer sequence

[00286] 5'-GGGACGGGGTGGGTAGACGGCGGGTATGTCCC -3'(SEQ ID NO 183), 5' PCR primer 5'-GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'-GGGACATACCCGCCG -3'(SEQ ID NO 184), were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGGACGGGGTGGGTAGACGGCGGGTATGTCCC -3' (SEQ ID NO 185).

[00287] The following seven minimized aptamer sequences were derived from the aptamer according to SEQ ID NO 49:

[00288] For minimized aptamer sequence 5'-

GGAGCGCACTCAGCCACACGACATTGGCGGGTTGTAATTACCACGCATGGCTG -3'(SEQ ID NO 186), 5' PCR primer 5'-GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'-CAGCCATGCGTGGTAATT-3' (SEQ ID NO 187), were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGAGCGCACTCAGCCACACGACATTGGCGGGTTGTAATTACCACGCATGGCTG -3'(SEQ ID NO188).

[00289] For minimized aptamer sequence 5'-

GGAGCCACACGACATTGGCGGGTTGTAATTACCACGCATGGCTCC-3' (SEQ ID NO 189), 5' PCR primer 5'-GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'-GGAGCCATGCGTGG-3' (SEQ ID NO190), were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGAGCCACACGACATTGGCGGGTTGTAATTACCACGCATGGCTCC-3' (SEQ ID NO191).

[00290] For minimized aptamer sequence 5'-

GGAGCCACACGACATTGGCGGGCGAGAGCCACGCATGGCTCC-3' (SEQ ID NO192), 5' PCR primer b 5'-GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'-GGAGCCATGCGTGG-3' (SEQ ID NO190) , were used to amplify template 5'-

GATCGATCTAATACGACTCACTATAGGAGCCACACGACATTGGCGGGCGAGAGCCACGCATGGCTCC-3'(SEQ ID NO 193).

[00291] For minimized aptamer sequence 5'-

GGAGCCACACGACATTGGCGAGAGCCACGCATGGCTCC-3'(SEQ ID NO 194), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'- GGAGCCATGCGTGG-3'(SEQ ID NO 190), were used to amplify template 5'- GATCGATCTAATACGACTCACTATAGGAGCCACACGACATTGGCGAGAGCCACG CATGGCTCC-3' (SEQ ID NO 195).

[00292] For minimized aptamer sequence 5'-

GGAGCCACACGAGAGTGGCGGGTTGTAATTACCACGCATGGCTCC-3'(SEQ ID NO 196), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3'(SEQ ID NO 166) and 3' PCR primer 5'- GGAGCCATGCGTGG-3' (SEQ ID NO 190), were used to amplify template 5'- GATCGATCTAATACGACTCACTATAGGAGCCACACGAGAGTGGCGGGTTGTAATT ACCACGCATGGCTCC-3' (SEQ ID NO 197).

[00293] For minimized aptamer sequence 5'-

GGCCACACGACATTGGCGGGCGAGAGCCACGCATGGCC-3' (SEQ ID NO 198), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'- GGCCATGCGTGGCTCTC -3'(SEQ ID NO 199), were used to amplify template 5'- GATCGATCTAATACGACTCACTATAGGCCACACGACATTGGCGGGCGAGAGCCA CGCATGGCC-3'(SEQ ID NO 200).

[00294] For minimized aptamer sequence 5'-

GGAGCCACACGACATTGGCGCGAGAGCGCATGGCTCC-3'(SEQ ID NO 201), 5' PCR primer 5'- GATCGATCTAATACGACTCACTATA -3' (SEQ ID NO 166) and 3' PCR primer 5'- GGAGCCATGCGCTCTCG -3'(SEQ ID NO 202), were used to amplify template 5'- GATCGATCTAATACGACTCACTATAGGAGCCACACGACATTGGCGCGAGAGCGC ATGGCTCC-3'(SEQ ID NO 203).

rRdY vWF Minimer Binding

[00295] All minimer sequences were transcribed, gel-purified on 15% denaturing polyacrylamide gels, 5-³²P end-labeled with γ^{32} P ATP, and then desalted using two Centri-Spin 10 columns (Princeton Separations Cat. # CS-101, Adelphia, NJ). For K_D determination, minimer transcripts were tested for direct binding to full length human vWF (SEQ ID NO 7), human vWF A1 domain (SEQ ID NO 5), and rabbit vWF A1 domain (SEQ ID NO 6) using an 8 point protein titration from 0-300 nM (3 fold dilutions) in 1X Dulbecco's PBS containing 0.1 mg/mL BSA (in a final volume of 50 μ L) for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell, Keene, NH). K_D values were calculated by fitting the equation $y = \frac{(\max/(1+K/\text{protein})) + y_{\text{int}}}{1 + (K/\text{protein})}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding characterization are tabulated in Table 18.

Table 18: rRdY aptamer minimer binding data, only aptamers that showed potent activity in cellular assays had their binding affinity measured. (ND = not done)

#	Minimer	Full length human vWF K_D (nM)	Human vWF A1 domain K_D (nM)	Rabbit vWF A1 domain K_D (nM)
1	SEQ ID NO 177	ND	ND	ND
2	SEQ ID NO 180	1	11	14
3	SEQ ID NO 183	10 ± 5	ND	14 ± 7
4	SEQ ID NO 186	ND	ND	ND
5	SEQ ID NO 189	1	ND	ND
6	SEQ ID NO 192	2 ± 0.2	4 ± 1	8 ± 1
7	SEQ ID NO 194		ND	
8	SEQ ID NO 196	ND	ND	ND
9	SEQ ID NO 198	3 ± 0.6	5 ± 2	11 ± 2

10	SEQ ID NO 201	ND	ND	ND
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EXAMPLE 2C: Truncation of DNA SELEX™ #1 vWF Aptamer

[00296] On the basis of the vWF binding analysis described in Example 1 above, as well as visual inspection of predicted folds, a panel of minimized sequences was designed for the best class of binders from Family #1. In this case all of the binders from Family #1 are structurally related and fell into one of four mutually exclusive folds (predicted by RNAstructure 4.1) as determined by the base-pairing constraints put on the 5'- and 3'-ends of the molecules. We synthesized and tested each of the four predicted folds. The sequence for each of the synthesized minimized DNA aptamers is as follows:

SEQ ID NO 204

5'-CCAGCGGAATGAGAATGCTGATGGATTGCTCAGGTCTGCTGG -3'

ARC 845 (SEQ ID NO 205)

5' ATGAGAGTGCTGGTGGATTGCTCAGGTCTGCTGGCTGCTTACTCTCAT -3'

SEQ ID NO 206

5'- CGATCTGACTAGCGGAATGAGAATGCTGGTGGATCG -3'

SEQ ID NO 207

5'- GATCTGACTAGCGCAATGAGGATGCdTGATGGATTGCTCAGGTC -3'

[00297] All minimizer DNA sequences were chemically synthesized, 5-³²P end-labeled with γ -³²P ATP, and then desalted using two Centri-Spin 10 columns (Princeton Separations, Cat.# CS-101, Adelphia, NJ). For K_D determination, minimizers were tested for binding to human vWF A1 domain (SEQ ID NO 5) using a competition dot blot assay with a constant protein concentration of 10 nM and a 12 point cold competitor DNA titration (3 uM, 1 uM, 333 nM, 100 nM, 33 nM, 10 nM, 3.3 nM, 1 nM, 333 pM, 100 pM, 33.3 pM, 0 pM) in 1X Dulbecco's PBS containing 0.1 mg/mL BSA (final volume of 50 uL) for 30 minutes at room temperature. K_D values were calculated by fitting the equation $y = (\max / (1 + K / \text{protein})) + y_{\text{int}}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding

characterization are tabulated in Table 19 below. As shown, only ARC845 of the minimized constructs retained the ability to bind to either human or rabbit vWF A1 domain.

Table 19. DNA 1 Minimer Binding Data (ND = Not Done)

#	Minimer	Full length human vWF K_D (nM)	Human vWF A1 domain K_D (nM)	Rabbit vWF domain K_D (nM)
1	ARC845 = SEQ ID NO 205	No binding	10	
2	SEQ ID NO 204	ND	No binding	No bi
3	SEQ ID NO 206	ND	No binding	
4	SEQ ID NO 207	ND	No binding	

[00298] Based on these binding results, ARC 845 (SEQ ID NO 205) represents the core nucleic acid binding sequence of the DNA SELEX™ 1, Family 1 aptamers.

EXAMPLE 2D: DNA vWF Alternating Selection Aptamer Minimization

[00299] On the basis of the vWF binding analysis described in Example 1 above and cell based assay data described in Example 3 below as well as visual inspection of predicted folds for aptamers AMX237.B11 (SEQ ID NO 109) and AMX236.A12 (SEQ ID NO 127), a series of minimized sequences were designed. Additionally, based on the observation that aptamers AMX237.G6 (SEQ ID NO 114), AMX238.E9 (SEQ ID NO 115), and AMX238.H5 (SEQ ID NO 118) appeared to be slightly more potent in cellular assays, a series of minimized sequences ARC1027-1031 (SEQ ID NOS 212-216) were synthesized. The minimized sequences according to SEQ ID NO 208 and SEQ ID NO 209 represent two mutually exclusive folds predicted from the full length aptamer AMX237.B11 (SEQ ID NO 109).

[00300] The nucleic acid sequences for above-described minimized DNA aptamers are as follows:

(SEQ ID NO 208)

5'- GGACGATCTGACTAGCTCCAGTGTTTTATCTAATAACCGTCC -3'

(SEQ ID NO 209)

5'- GGAGCTCCAGTGTTTTATCTAATAACCGTGCGGTGCCTCCGTGAGCTCC -3'

(SEQ ID NO 210)

5'- GGAGCTGCGGTTTGGTCGACGTCAGCTCC -3'

(SEQ ID NO 211)

5'- GGTAGCGACTCTGTGGAGCTGCGGTTTGG -3'

ARC1027 (SEQ ID NO 212)

5'-GGCGTGCAAGTGCCTATTCTAGGCCGTGCGGTGCCTCCGTCACGCC-3T-3'

ARC1028 (SEQ ID NO 213)

5'-dGCGTGCAAGTGCCT-[PEG]-AGGCCGTGCGGTGCCTCCGTCACGCC-3T-3'

ARC1029 (SEQ ID NO 214)

5'-GGCGTGCAAGTGCC-[PEG]-GGCCGTGCGGTGCCTCCGTCACGCC-3T -3'

ARC1030 (SEQ ID NO 215)

5'- GGCGTGCAAGTGCCTATTCTAGGCCGTGCGG-[PEG]-CCGTCACGCC-3T -3'

ARC1031 (SEQ ID NO 216)

5'- GGCGTGCAAGTGCCT-[PEG]-AGGCCGTGCGG-[PEG]-CCGTCACGCC-3T -3'

[00301] All of the above minimized aptamer sequences were chemically synthesized , gel-purified on 15% denaturing polyacrylamide gels and then desalted using two Centri-Spin 10 columns (Princeton Separations Cat.# CS-101, Adelphia, NJ) using standard methods and techniques. The minimized sequences were characterized in cellular assays as described in Example 3 below.

[00302] Of the initial series, SEQ ID NO 208 to SEQ ID NO 211, only SEQ ID NO 208 demonstrated activity in the cellular assays (see Example 3, below). Comparison of the sequences of aptamers AMX237.B11 (SEQ ID NO 109) and AMX237.G6 (SEQ ID NO 114), AMX238.E9 (SEQ ID NO 115), and AMX238.H5 (SEQ ID NO 118) revealed them to be closely related and to support the predicted secondary structure of the minimized aptamer (SEQ ID NO 208) (see Figures 14 and 15). These molecules, ARC1027-1031 (SEQ ID NOS 212-216) further tested our hypothesis about the folding and secondary structure of aptamers AMX237.G6 (SEQ ID NO 114), AMX238.E9 (SEQ ID NO 115), and AMX238.H5 (SEQ ID NO 118) (see Figures 5, 14 and 15).

[00303] For K_D determination, the minimized sequences that showed potent activity in the cellular assays as described in Example 3 below were 5- 32 P end-labeled with γ - 32 P ATP, and then desalted using two Centri-Spin 10 columns (Princeton Separations Cat.# CS-101, Adelphia, NJ). Minimizers were tested for direct binding to full length human vWF (SEQ ID NO 7), and rabbit vWF A1 domain (SEQ ID NO 6) using a 9 point protein titration (100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 0 pM) (see Figure 6) in 1X Dulbecco's PBS containing 0.1 mg/mL BSA (final volume of 50 μ L) for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell, Keene, NH). K_D values were calculated by fitting the equation $y = (\max/(1+K/\text{protein})) + y_{\text{int}}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding characterization are tabulated in Table 20.

Table 20. DNA 2 aptamer minimizer binding data, only aptamer minimizers that showed potent activity in cellular assays had their binding affinity measured (see Example 3 below) ('ND' = not done)

#	Minimer	Full length human vWF K_D (nM)	Human vWF A1 domain K_D (nM)	Rabbit vWF A1 domain K_D (nM)
1	SEQ ID NO 208	ND	ND	ND
2	SEQ ID NO 209	ND	ND	ND
3	SEQ ID NO 210	ND	ND	ND
4	SEQ ID NO 211	ND	ND	ND
5	ARC 1027 (SEQ ID NO 212)	0.8	ND	4.6
6	ARC1028 (SEQ ID NO 213)	1.1	ND	3.8
7	ARC1029 (SEQ ID NO 214)	1.4 ± 0.2	ND	6.5 ± 1.5
8	ARC1030 (SEQ ID NO 215)	No binding	ND	No binding
9	ARC1031 (SEQ ID NO 216)	No binding	ND	No binding

EXAMPLE 2E: Optimization of ARC1029 Through Aptamer Medicinal Chemistry

[00304] Highly stable and potent variants of ARC1029 (SEQ ID NO 214) were identified through a systematic synthetic modification approach involving 5 phases of aptamer synthesis, purification and assay for binding activity. To facilitate the ease of chemical synthesis during aptamer modification, the PEG spacer of ARC1029 (SEQ ID NO 214) was replaced with a short oligonucleotide sequence, dTdTdC, resulting in ARC1115 (SEQ ID NO 221) as seen in Figure 16 and Table 21 below. A highly stabilizing 3'-inverted dT was synthesized on the three prime end of ARC1115 (SEQ ID NO 221) resulting in ARC1172 (SEQ ID NO 222) (SEQ ID NO 222) also as seen in Figure 16 and Table 21 below. Once both ARC1115 (SEQ ID NO 221) and ARC1172 (SEQ ID NO 222) (SEQ ID NO 222) had been shown to bind to human vWF, ARC1172 (SEQ ID NO 222) (SEQ ID NO 222) was used as the basic template for modification as described in the Examples below.

[00305] In phase 1 of the modification process, each individual residue in ARC1172 (SEQ ID NO 222) was replaced by the corresponding 2'-O methyl containing residue (with dT being replaced by mU unless otherwise specified) resulting in ARC1194 (SEQ ID NO 223) – ARC1234 as shown in Table 21 below and Figure 16. Additionally in phase 1, a set of composite replacements were made in the stem regions of ARC1172 (SEQ ID NO 222) resulting in ARC1235 to 1243 also as shown in Table 21 and in Figure 16.

[00306] As described herein, see *e.g.*, in Examples 1, 2, and 3, during the processes of clone screening and truncation that led to ARC1029 (SEQ ID NO 214), there was excellent agreement among the relative potency of aptamers in binding (dot-blot), FACS and BIPA assays. Accordingly, affinity for full length human vWF measured as measured in dot-blot assay binding assays was used to characterize relative affinity of the majority of the aptamer test variants synthesized.

[00307] For K_D determination, chemically synthesized aptamers were purified using denaturing polyacrylamide gel electrophoresis, 5' end labeled with γ -³²P ATP and were tested for direct binding to full length human vWF (Calbiochem Cat.# 681300, La Jolla, CA). An 8 point protein titration was used in the dot blot binding assay (100n M, 30 nM, 10 nM, 3 nM, 1

nM, 300 pM, 100 pM, 0 pM)) in 1X Dulbecco's PBS containing 0.1 BSA (final volume of 50 uL) for 30 minutes at room temperature. K_D values were calculated by fitting the equation $y = (\text{max}/(1+K/\text{protein})) + y_{\text{int}}$ using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Sequences of the ARC1029 (SEQ ID NO 214) derivatives synthesized, purified and assayed for binding to full length human vWF as well as the results of the protein binding characterization are tabulated Table 21 below, Binding affinity (K_D) is presented in the fourth column and extent of aptamer binding at 100 nM vWF is presented in the final column of Table 21.

Table 21: Phase 1 Modification Binding Results

SEQ ID NO:	ARC #	Sequence (5' -> 3'), (NH ₂ = 5'-hexylamine linker phosphoramidite), (3T = inv dT), (T=dT), (s=phosphorothioate), (mN = 2'-O Methyl containing residue), (PEG = polyethylene glycol), (dN=deoxy residue)	K_D (nM)	% binding @ 100nM vWF
221	ARC1115	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC	2	36
222	ARC1172	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	37
223	ARC1194	mGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	3	46
224	ARC1195	dGmGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	1	55
225	ARC1196	dGdGmCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	46

226	ARC1197	dGdGdCmGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	46
227	ARC1198	dGdGdCdGmUdGdCdAdGTdGdC dCTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	0.9	50
228	ARC1199	dGdGdCdGTmGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	19	28
229	ARC1200	dGdGdCdGTdGmCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	5	25
230	ARC1201	dGdGdCdGTdGdCmAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	0.9	6
231	ARC1202	dGdGdCdGTdGdCdAmGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	0.4	56
232	ARC1203	dGdGdCdGTdGdCdAdGmUdGdC dCTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	3	40
233	ARC1204	dGdGdCdGTdGdCdAdGTmGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	45
234	ARC1205	dGdGdCdGTdGdCdAdGTdGmCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	1	50
235	ARC1206	dGdGdCdGTdGdCdAdGTdGdCm CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	50

236	ARC1207	dGdGdCdGTdGdCdAdGTdGdCd CmUTdCdGdGdCdCdGTdGdCdG dGTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	54
237	ARC1208	dGdGdCdGTdGdCdAdGTdGdCd CTmUdCdGdGdCdCdGTdGdCdG dGTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	45
238	ARC1209	dGdGdCdGTdGdCdAdGTdGdCd CTTmCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	44
239	ARC1210	dGdGdCdGTdGdCdAdGTdGdCd CTTdCmGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	49
240	ARC1211	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGmGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	47
241	ARC1212	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGmCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	49
242	ARC1213	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCmCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	6	43
243	ARC1214	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCmGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	3	39
244	ARC1215	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGmUdGdCdG dGTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	3	2
245	ARC1216	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGmGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	0.6	40

246	ARC1217	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGmCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	14	18
247	ARC1218	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCmGd GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	32	2
248	ARC1219	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGm GTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	3	33
249	ARC1220	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GmUdGdCdCTdCdCdGTdCdAdC dGdCdC-3T	11	17
250	ARC1221	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTmGdCdCTdCdCdGTdCdAdCd GdCdC-3T	1	43
251	ARC1222	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGmCdCTdCdCdGTdCdAdCd GdCdC-3T	0.9	40
252	ARC1223	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCmCTdCdCdGTdCdAdCd GdCdC-3T	36	26
253	ARC1224	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCmUdCdCdGTdCdAdC dGdCdC-3T	0.5	47
254	ARC1225	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTmCdCdGTdCdAdCd GdCdC-3T	11	16
255	ARC1226	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCmCdGTdCdAdCd GdCdC-3T	12	25

256	ARC1227	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCmGTdCdAdCd GdCdC-3T	3	40
257	ARC1228	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGmUdCdAdC dGdCdC-3T	2	43
258	ARC1229	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTmCdAdCd GdCdC-3T	5	37
259	ARC1230	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCmAdCd GdCdC-3T	3	46
260	ARC1231	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAmCd GdCdC-3T	1	50
261	ARC1232	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCm GdCdC-3T	1	51
262	ARC1233	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GmCdC-3T	2	39
263	ARC1234	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCdGd GTdGdCdCTdCdCdGTdCdAdCd GdCmC-3T	2	42
264	ARC1235	mGmGdCmGTmGdCdAdGTdGd CdCTTdCdGdGdCdCdGTdGdCd GdGTdGdCdCTdCdCdGTdCmAd CmGdCdC-3T	13	23
265	ARC1236	dGdGmCdGmUdGdCdAdGTdGd CdCTTdCdGdGdCdCdGTdGdCd GdGTdGdCdCTdCdCdGTmCdA mCdGmCmC-3T	3	32

266	ARC1237	mGmGmCmGmUmGdCdAdGTd GdCdCTTdCdGdGdCdCdGTdGd CdGdGTdGdCdCTdCdCdGTmCm AmCmGmCmC-3T	41	9
267	ARC1238	dGdGdCdGTdGdCdAdGTmGdCd CTTdCmGmGdCdCdGTdGdCdG dGTdGdCdCTdCdCdGTdCdAdCd GdCdC-3T	2	43
268	ARC1239	dGdGdCdGTdGdCdAdGTdGmC mCTTdCdGdGmCdCdGTdGdCd GdGTdGdCdCTdCdCdGTdCdAd CdGdCdC-3T	5	37
269	ARC1240	dGdGdCdGTdGdCdAdGTmGmC mCTTdCmGmGmCdCdGTdGdCd GdGTdGdCdCTdCdCdGTdCdAd CdGdCdC-3T	4	40
270	ARC1241	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGdCmG mGTdGdCdCTdCdCmGTdCdAdC dGdCdC-3T	no binding	no binding
271	ARC1242	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGmCdGd GTdGdCdCTmCmCdGTdCdAdCd GdCdC-3T	no binding	no binding
272	ARC1243	dGdGdCdGTdGdCdAdGTdGdCd CTTdCdGdGdCdCdGTdGmCmG mGTdGdCdCTmCmCmGTdCdAd CdGdCdC-3T	no binding	no binding

[00308] As can be seen from the binding data in Table 21, the positions that most readily tolerate substitution of a deoxy residue for a 2'-O methyl residue correlate well with the sequence conservation mapped onto the secondary structure of ARC1029 (SEQ ID NO 214 shown in Figure 15 thus providing further, independent support for the proposed structure of the aptamer. The positions of ARC1172 (SEQ ID NO 222) that do not tolerate 2'-O-Me modifications as well as the positions that do are shown in Figure 15B.

[00309] Based upon the structure activity relationship (SAR) results of the individual and composite deoxy to methoxy aptamers described immediately above in the phase 1

modification process, a second series of aptamers was designed, synthesized, purified and tested for binding to vWF. For these and all subsequent aptamers, molecules that retained an affinity (K_D) of ~ 10 nM or better as well as an extent of binding at 100 nM vWF of at least 35% were the goal. ARC1338 (SEQ ID NO 273)-ARC1348 (SEQ ID NO 283), as shown in Figure 17 and Table 21, were synthesized during phase 2 of the modification process. ARC1338 (SEQ ID NO 273) to 1342 were synthesized with block modifications based on the tolerated individual substitutions from phase 1 modification. ARC1343 (SEQ ID NO 278)-ARC1345 were synthesized each with a different phosphorothioate phosphate backbone modification (see Figure 17 and Table 22 below) Lastly, ARC1346 (SEQ ID NO 281)-ARC1348 (SEQ ID NO 283) were synthesized to test removing a single base pair from stem 1, stem 2 and from both stems of ARC1342 as shown in Figure 17 and Table 22 below.

Table 22: Phase 2 Modification Binding Results

SEQ ID NO :	ARC #	Sequence (5' -> 3'), (NH ₂ = 5'-hexylamine linker phosphoramidite), (3T = inv dT), (T=dT), (s=phosphorothioate), (mN = 2'-O Methyl containing residue), (PEG = polyethylene glycol), (dN=deoxy residue)		
			K_D (nM)	% binding @ 100nM vWF
273	ARC1338	mGmGmCmGmUdGdCdAdGTdGdCdCTTdCdGdGdCdCdGTdGdCdGdGTdGdCdCTdCdCdGTdCmAmCmGmCmC-3T	7.4	21
274	ARC1339	dGdGdCdGTdGdCdAmGmUdGdCdCTTdCdGdGdCdCmGTmGdCdGdGTdGdCdCTdCdCmGmUmCdAdCdGdCdC-3T	2.4	39
275	ARC1340	dGdGdCdGTdGdCdAdGTdGdCdCTTdCdGdGdCdCdGTmGdCdGdGTmGmCdCmUdCdCmGmUmCdAdCdGdCdC-3T	7.4	43
276	ARC1341	mGmGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCdGTdGdCdGdGTdGdCdCTdCdCdGTdCmAmCmGmCmC-3T	22.5	26

277	ARC1342	mGmGmCmGmUdGdCdAmGmUm GmCmCmUmUmCmGmGmCdCm GTmGdCdGdGTmGmCdCmUdCd CmGmUmCmAmCmGmCmC-3T	15.6	33
278	ARC1343	mGmGmCmGmUdG-s- dCdAmGmUmGmCmCmUmUmC mGmGmC-s-dCmGTmGdCdG-s- dGTmGmCdCmUdC-s- dCmGmUmCmAmCmGmCmC-3T	23.9	21
279	ARC1344	mGmGmCmGmUdG-s-dC-s- dAmGmUmGmCmCmUmUmCmG mGmC-s-dCmGTmGdC-s-dG-s- dGTmGmCdCmUdC-s- dCmGmUmCmAmCmGmCmC-3T	4.8	17
280	ARC1345	mGmGmCmGmU-s-dG-s-dC-s- dAmGmUmGmCmCmUmUmCmG mGmC-s-dCmG-s-TmG-s-dC-s-dG- s-dG-s-TmGmC-s-dCmU-s-dC-s- dCmGmUmCmAmCmGmCmC-3T	12.1	29
281	ARC1346	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmUdCdCm GmUmCmAmCmGmC-3T	11	51
282	ARC1347	mGmGmCmGmUdGdCdAmGmUm GmCmUmUmCmGmCdCmGTmGd CdGdGTmGmCdCmUdCdCmGmU mCmAmCmGmCmC-3T	no binding	no binding
283	ARC1348	mGmCmGmUdGdCdAmGmUmGm CmUmUmCmGmCdCmGTmGdCd GdGTmGmCdCmUdCdCmGmUm CmAmCmGmC-3T	no binding	no binding

[00310] As seen in Table 22, the results from phase 2 of aptamer modification revealed ARC1346 (SEQ ID NO 281) to be the most potent of the highly substituted ARC1029 (SEQ ID NO 214) derivative aptamers generated thus far. Interestingly as shown by the results with ARC1347 (SEQ ID NO 282) and ARC1348 (SEQ ID NO 283), removal of a base pair from stem 2 is not tolerated in this highly modified context.

[00311] ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), shown in Table 23 and Figure 17, were synthesized during phase 3 of the aptamer modification process. As the dG to mG substitution at position 6 was poorly tolerated in test variants in phase 1 aptamer modification and guanosine at position 6 pairs with the cytidine at position 36, ARC1346 (SEQ ID NO 281) was synthesized with a mC to dC modification at position 36 resulting in ARC1361 (SEQ ID NO 284) as shown in Table 23 below. ARC1361 (SEQ ID NO 284) served as the base sequence for introduction of single phosphorothioate phosphate backbone modifications that resulted in ARC1362 to ARC1381 (SEQ ID NO 304) also shown in Table 23 below.

Table 23: Phase 3 Modification Binding Results

SEQ ID NO :	ARC #	Sequence (5' -> 3'), (NH ₂ = 5'-hexylamine linker phosphoramidite), (3T = inv dT), (T=dT), (s=phosphorothioate), (mN = 2'-O Methyl containing residue), (PEG = polyethylene glycol), (dN=deoxy residue)	K _D (nM)	% binding @ 100nM vWF
222	ARC1172	dGdGdCdGTdGdCdAdGTdGdCdC TTdCdGdGdCdCdGTdGdCdGdGT dGdCdCTdCdCdGTdCdAdCdGdCd C-3T	2	37
284	ARC1361	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmUdCdCm GmUdCmAmCmGmC-3T	7.9	38.5
285	ARC1362	mGmCmGmU-s- dGdCdAmGmUmGmCmCmUmUm CmGmGmCdCmGTmGdCdGdGT mGmCdCmUdCdCmGmUdCmAm CmGmC-3T	9.9	34.3
286	ARC1363	mGmCmGmUdG-s- dCdAmGmUmGmCmCmUmUmC mGmGmCdCmGTmGdCdGdGTm GmCdCmUdCdCmGmUdCmAmC mGmC-3T	12.7	32.7

287	ARC1364	mGmCmGmUdGdC-s- dAmGmUmGmCmCmUmUmCmG mGmCdCmGTmGdCdGdGTmGm CdCmUdCdCmGmUdCmAmCmG mC-3T	8.2	36.9
288	ARC1365	mGmCmGmUdGdCdA-s- mGmUmGmCmCmUmUmCmGmG mCdCmGTmGdCdGdGTmGmCdC mUdCdCmGmUdCmAmCmGmC- 3T	10.8	35.4
289	ARC1366	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmC-s- dCmGTmGdCdGdGTmGmCdCmU dCdCmGmUdCmAmCmGmC-3T	15.5	28.9
290	ARC1367	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdC-s- mGTmGdCdGdGTmGmCdCmUdC dCmGmUdCmAmCmGmC-3T	13.9	30.4
291	ARC1368	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmGmC-3T	1.8	38.2
292	ARC1369	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT- s- mGdCdGdGTmGmCdCmUdCdCm GmUdCmAmCmGmC-3T	16.3	26.2
293	ARC1370	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mG-s- dCdGdGTmGmCdCmUdCdCmGm UdCmAmCmGmC-3T	10.1	22.5
294	ARC1371	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdC-s- dGdGTmGmCdCmUdCdCmGmUd CmAmCmGmC-3T	8.4	32.1
295	ARC1372	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdG-s- dGTmGmCdCmUdCdCmGmUdCm AmCmGmC-3T	23.5	35.2

296	ARC1373	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdG-s- TmGmCdCmUdCdCmGmUdCmA mCmGmC-3T	7.1	33.0
297	ARC1374	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGT-s- mGmCdCmUdCdCmGmUdCmA CmGmC-3T	9.5	27.2
298	ARC1375	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmC-s- dCmUdCdCmGmUdCmA CmGmC-3T	8.8	25.5
299	ARC1376	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdC-s- mUdCdCmGmUdCmA CmGmC-3T	4.4	31.3
300	ARC1377	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmU-s- dCdCmGmUdCmA CmGmC-3T	7.4	30.9
301	ARC1378	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmUdC-s- dCmGmUdCmA CmGmC-3T	9.1	31.1
302	ARC1379	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmUdCdC-s- mGmUdCmA CmGmC-3T	10.4	31.3
303	ARC1380	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmUdCdCm GmU-s-dCmA CmGmC-3T	12.0	32.5
304	ARC1381	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGT mGdCdGdGTmGmCdCmUdCdCm GmUdC-s-mA CmGmC-3T	8.7	35.8

[00312] As shown in Table 23 above, while the majority of the modifications tested in phase 3 had little or no beneficial effect, ARC1368 (SEQ ID NO 291), which contains a single phosphorothioate modification between mG-20 and dT-21 binds to human vWF with an affinity identical (within experimental error) to that of the parent compound, ARC1172 (SEQ ID NO 222).

[00313] During phase 4 and phase 5 aptamer modification, ARC1524 (SEQ ID NO 305) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317) and ARC1759 (SEQ ID NO 318), shown in Figure 18, were synthesized. A circular permutation of the sequence that closed stem 1 and opened stem 2 as illustrated in Figure 19 was synthesized.

[00314] As shown in Table 24 below, many of these aptamers bound to vWF, however, none were as potent as ARC1368 (SEQ ID NO 291). Interestingly, though consistent with the SAR generated in Phase 1 of aptamer modification, ARC1525, containing only a single change from dT to mT at position 27, showed no binding at all to vWF. ARC1525 was used as a negative control in many of the biological assays in which ARC1368 (SEQ ID NO 291) was subsequently tested. Again, consistent with the SAR data from Phase 3 of aptamer modification, ARC1759 (SEQ ID NO 318) which is identical to ARC1172 (SEQ ID NO 222) except that it has single phosphorothioate substitution between the G at position 21 and the T at position 22 showed measurable improvement in affinity relative to ARC1172 (SEQ ID NO 222).

Table 24: Phase 4 and 5 Aptamer Modification Binding Results

SEQ ID NO:	ARC #	Sequence (5' -> 3'), (NH ₂ = 5'-hexylamine linker phosphoramidite), (3T = inv dT), (T=dT), (s=phosphorothioate), (mN = 2'-O Methyl containing residue), (PEG = polyethylene glycol), (dN=deoxy residue)	K _D (nM)	% binding @ 100nM vWF
222	ARC1172	dGdGdCdGTdGdCdAdGTdGdCdC TTdCdGdGdCdCdGTdGdCdGdGT dGdCdCTdCdCdGTdCdAdCdGdCd C-3T	2	37
291	ARC1368	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmGmC-3T	1.8	38.2
305	ARC1524	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmGmC-3T	5.7	26.5
306	ARC1525	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmGm TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmGmC-3T	No binding	No binding
307	ARC1526	mGmCmGmUdGdCdAmGmUmGm CmCmUmUmUmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmGmC-3T	4.7	29.0
308	ARC1527	mGmCmGmUdGdCdAmGmUmGm CmCPEGmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmGmC-3T	3.4	14.1
309	ARC1528	mGmCmGmUdGdCdAmGmUmGm CmCPEGmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmGmC-3T	4.2	10.2
310	ARC1529	mCmGmUdGdCdAmGmUmGmCm CmUmUmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmG-3T	12.3	22.6

311	ARC1530	mCmGmUdGdCdAmGmUmGmCm CmUmUmUmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmG-3T	15.0	19.2
312	ARC1531	mCmGmUdGdCdAmGmUmGmCm CPEGmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmG-3T	1.5	20.0
313	ARC1532	mCmGmUdGdCdAmGmUmGmCm CPEGmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmG-3T	2.6	19.3
314	ARC1533	mCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCPEGmGmUdGdC dAmGmUmGmCmCmG-3T	4.0	31.7
315	ARC1534	mCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmUmUmUmGm UdGdCdAmGmUmGmCmCmG-3T	62.8	25.6
316	ARC1535	mCmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUmCmAmCmUmUmUmGm UdGdCdAmGmUmGmCmCmGmG -3T	27.1	48.3
317	ARC1546	mCmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmGmUmUmCmC mGmUdGdCdAmGmUmGmCmCm GmG-3T	24	26

318	ARC1759	dGdGdCdGTdGdCdAdGTdGdCdC TTdCdGdGdCdCdG-s- TdGdCdGdGTdGdCdCTdCdCdGT dCdAdCdGdCdC-3T	0.7	46
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EXAMPLE 2F: Conjugation of PEG moieties to modified aptamers

[00315] Polyethylene glycol moieties were conjugated to the 5' terminus of ARC1368 (SEQ ID NO 291) and ARC1172 (SEQ ID NO 222) via amine reactive chemistries. The oligonucleotides ARC1635 NH₂-mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmG-s-TmGdCdGdGdGdGmCdCmUdCdCmGmUdCmAmCmGmC-3T (ARC1368 (SEQ ID NO 291) with a 5' hexylamine modification) and ARC1884 (SEQ ID NO 322) NH₂-dGdGdCdGdGdGdCdAdGdGdGdCdCTdGdCdGdGdCdCdGTdGdCdGdGTdGdCdCTdCdCdGTdCdAdCdGdCdC-3T (ARC1172 (SEQ ID NO 222) with a 5' hexylamine modification) were chemically synthesized.

[00316] The amine-modified aptamers were conjugated to different PEG moieties, as indicated in Table 25 below, post-synthetically.

[00317] Table 25: Hexylamine modified or PEG conjugated aptamers

SEQ ID NO:	ARC #	Sequence (5' -> 3'), (NH ₂ = 5'-hexylamine linker phosphoramidite), (3T = inv dT), (T=dT), (s=phosphorothioate), (mN = 2'-O Methyl containing residue), (PEG = polyethylene glycol), (dN= deoxy residue)
319	ARC1635	NH ₂ -mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmG-s-TmGdCdGdGTmGmCdCmUdCdCmGmUdCmAmCmGmC-3T

320	ARC1779	PEG20K-NH2- mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmGmC-3T
321	ARC1780	PEG40K-NH2- mGmCmGmUdGdCdAmGmUmGm CmCmUmUmCmGmGmCdCmG-s- TmGdCdGdGTmGmCdCmUdCdC mGmUdCmAmCmGmC-3T
322	ARC1884	NH2- dGdGdCdGTdGdCdAdGTdGdCdC TTdCdGdGdCdCdGTdGdCdGdGT dGdCdCTdCdCdGTdCdAdCdGdCd C-3T
323	ARC1885	PEG20K-NH2- dGdGdCdGTdGdCdAdGTdGdCdC TTdCdGdGdCdCdGTdGdCdGdGT dGdCdCTdCdCdGTdCdAdCdGdCd C-3T

EXAMPLE 3: FUNCTIONAL CELL ASSAYS

Biological vWF Dependent Assays

[00318] The effectiveness of various aptamers in blocking vWF function in several biological assays is described in this Example.

[00319] In one assay botrocetin is used. Botrocetin, a protein isolated from snake venom, is known to induce von Willebrand Factor binding to the gpIb receptor on live and fixed platelets. This reaction causes agglutination of suspensions of fixed platelets via vWF multimerization. In preparations of platelet rich plasma (hereinafter "PRP"), vWF/botrocetin induction of agglutination is followed by a second phase of platelet aggregation caused by metabolic activation of the platelets. These two reactions: vWF binding to fixed platelets and vWF mediated platelet aggregation, can be used to measure the activity of aptamers of the invention.

[00320] The amount of vWF bound to fixed platelets can be measured with an antibody to vWF. The fluorescence signal from bound antibody incubated with a fluorescein conjugated secondary antibody is then detected and quantified by flow cytometry. The ability of an aptamer of the invention to block vWF binding to platelets is correlated with a reduction in fluorescence signal.

[00321] Botrocetin induces the binding of the A1 domain of vWF to platelets, as well as the full length protein. It was determined by the inventors that 6-Histidine-tagged rabbit A1 domain vWF purified protein could be induced to bind to human lyophilized platelets with botrocetin. Rabbit A1 binding to platelets is measured with an anti-poly-His antibody followed by incubation with a phycoerythrin conjugated secondary antibody. The degree of binding can be quantified by flow cytometric analysis. The ability of aptamers to block the binding of rabbit A1 to human fixed platelets was correlated with decreased fluorescence signal.

[00322] In platelet rich plasma isolated from fresh human blood, botrocetin induces platelet aggregation via vWF. Platelet aggregate formation can be measured optically as an increase in % light transmittance on a Chronolog Model 490-4D Aggregometer because aggregation of platelets clarifies the plasma. Aptamers of the invention were analyzed for their ability to inhibit botrocetin induced platelet aggregation ("BIPA") in human blood. An aptamer of the invention was considered to be active if it could prevent aggregate formation for six minutes post botrocetin addition.

[00323] Another assay of this Example, using the PFA-100 instrument, is an agonist independent but vWF dependent assay that uses the PFA-100 instrument (Harrison *et al.*, Clin. Lab. Haem., v24, p225-32 (2002)). The PFA-100 simulates the formation of a hemostatic plug under conditions of high shear force *in vivo* by recording the time required for a platelets to aggregate and block the flow of citrated whole blood through a microscopic aperture in a membrane coated with collagen and either epinephrine or ADP. This activity is von Willebrand factor dependent as high MW vWF multimers bind to immobilized collagen on the membrane and then bind to and activate platelets because of the shear force induced by drawing the blood through the microscopic aperture. Thus this assay is complimentary to the BIPA and FACS assays in that it is vWF dependent, however it has some advantages in that it

does not require the addition of the vWF agonist botrocetin and uses whole blood instead of platelet rich plasma.

[00324] Another assay of this Example used ADP to induce platelet aggregation. Aggregation of platelet rich plasma (PRP) can be induced in multiple ways. The snake venom protein botrocetin acts on vWF as described above, stabilizing its interaction with the platelet receptor gpIb thereby inducing platelet aggregation. Binding of vWF to gpIb is an early step in platelet aggregation, thus there is an expectation that inhibitors that block downstream components of the aggregation process (*i.e.*, the IIbIIIa antagonists Integrelin™ and ReoPro™) would also prevent botrocetin induced platelet aggregation. However, in the case of agonists that act directly on platelets and induce aggregation (ADP for example), one would expect that antagonists upstream of the agonist would be ineffective (an anti-vWF aptamer for example), while antagonists that act directly on platelets (IIbIIIa antagonists) would remain potent. The specificity of a vWF antagonist relative to a IIbIIIa antagonist will increase the safety of the anti-vWF antagonist by decreasing the bleeding time associated with treatment. For patients with atherosclerotic plaques in stenosed arteries, platelet aggregation occurs as platelets bind to collagen immobilized vWF on the surface of the plaque. Thus both inhibiting the vWF/gpIb interaction as well as blocking the IIbIIIa receptor binding to fibrin will prevent platelet aggregation. The biological specificity conferred by targeting vWF insures that unlike anti-IIbIIIa treatment, platelets themselves are not targeted directly insuring they can still be activated by other means, thus reducing potential bleeding complications associated with anti-platelet therapy.

[00325] The following materials were used in Examples 3A-3D described below: human von Willebrand Factor (vWF) (SEQ ID NO 7), and bovine serum albumin were purchased from Calbiochem (Cat# 681300 and #126593, respectively) (La Jolla, CA); domain A1 rabbit vWF (SEQ ID NO 6) was expressed and purified using standard methods and conditions. Lyophilized human platelets (P/N 299-2), cuvettes (P/N 312), stir bars (P/N 311), platelet aggregometer (model 490-4D), and AGGRO/LINK Software were purchased from Chronolog (Haverton, PA). Botrocetin (12201-100U-B) was manufactured by Pentapharm (Basel, Switzerland). Fresh blood was obtained from apparently healthy, nonsteroidal anti-inflammatory drug ("NSAID") free donors and was drawn into 5 mL 0.105 M Sodium Citrate

Vacutainer tubes (Cat# 369714) (Becton Dickinson- Franklin Lakes, NJ). Physiological saline was manufactured by Aldon (Cat # 9420306) (Avon, NY) and phosphate buffered saline (Cat # 21-040-CV) was purchased from Cellgro (Herndon, VA). Flow cytometric experiments were performed on a BD Biosciences FACSCAN machine and analyzed with CellQuest Software (San Jose, CA). Anti-von Willebrand Factor mouse monoclonal antibody (Cat# GTI-V1A) was purchased from GTI (Waukesha, WI). Penta-HIS-biotin conjugate monoclonal antibody (Cat # 34440) was purchased from Qiagen (Germany). Anti-mouse IgG2a – FITC conjugate (Cat # 553390) was purchased from BD Biosciences (San Diego, CA). Anti-mouse IgG-PE conjugate antibody (Cat # 715-116-150) was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

EXAMPLE 3A: Full Length Human von Willebrand Factor Platelet Binding Assay

[00326] Aptamer potency to block human vWF binding to lyophilized platelets was assessed by flow cytometric analysis. Titrations of aptamers (0 nM, 0.1 nM to 1000 nM) were pre-incubated briefly with 5 nM of full length human vWF in FACS buffer (PBS plus 0.5% bovine serum albumin) at room temperature in a volume of 50 uL. Another 50 uL containing 5 uL of lyophilized platelets plus 1 uL of 0.1 U/uL of botrocetin in FACS buffer was added to aptamer/vWF. This reaction was allowed to proceed for 15 minutes at 37 degrees C after which 200 uL of FACS buffer was added. Platelets were collected by a 6 minute spin at 1470 RCF and the supernatant was discarded. The pellets were resuspended in 100 uL of FACS buffer containing a 1:100 dilution of anti-vWF antibody and were incubated at room temperature for 30 minutes. After dilution with 200 uL of FACS buffer, the platelets were spun at 1470 RCF for 6 minutes and the supernatant was discarded. The pellets were resuspended in a 1:100 solution of anti-IgG2a-FITC antibody and were incubated in the dark for 30 minutes at room temperature. The entire 100 uL was diluted into 200 uL of FACS buffer and analyzed immediately by flow cytometric analysis in the FACSCAN. Artfactual data from contaminating debris was eliminated from the analysis by drawing a gate around the population of single and aggregated platelets. Mean fluorescent intensity ("MFI") readings were quantified for each sample analyzed by flow cytometry. Background MFI was subtracted from all data points. Percent inhibition was reported by calculating the percent value of binding of full length human vWF to platelets in the presence of aptamer at a given

concentration relative to binding in the absence of any aptamer (see Figure 7). IC_{50} values were determined by fitting the percent inhibition of vWF binding to platelets as a function of aptamer concentration to the equation:

$$\% \text{ inhibition} = \% \text{ inhibition}_{\text{max}} / (1 + IC_{50} / \text{aptamer conc.})$$

[00327] Results of botrocetin induced vWF binding characterization are tabulated in Table 26 below.

EXAMPLE 3B: Rabbit von Willebrand Factor Domain A1 Platelet Binding Assay

[00328] The ability of aptamers of the invention to block rabbit vWF domain A1 binding to lyophilized platelets was also assessed by flow cytometric analysis. Titrations of aptamers (zero, 0.1 nM to 1000 nM) were preincubated briefly with 4 nM of rabbit A1 vWF in FACS buffer (PBS plus 0.5% bovine serum albumin) at room temperature in a volume of 50 uL. Another 50 uL containing 5uL of lyophilized platelets plus 1uL of 0.1 U/uL of botrocetin in FACS buffer was added to aptamer/vWF. This reaction was allowed to proceed for 15 minutes at 37 degrees C after which 200 uL of FACS buffer was added. Platelets were collected by a 6 minute spin at 1470 RCF and the supernatant was discarded. The pellets were resuspended in 100uL of FACS buffer containing a 1:200 dilution of anti-Penta-HIS-biotin conjugate antibody and were incubated at room temperature for 30 minutes. After the dilution with 200uL of FACS buffer, the platelets were spun at 1470 RCF for 6 minutes, and the supernatant was discarded. The pellets were resuspended in a 1:100 solution of anti-IgG-PE antibody and were incubated in the dark for 30 minutes at room temperature. The entire 100 uL was diluted into 200 uL of FACS buffer and analyzed immediately by flow cytometric analysis in the FACSCAN. Contaminating debris was eliminated from the analysis by drawing a gate around the population of single and aggregated platelets and collecting data from 10000 events. Median fluorescent intensity ("MedFI") readings (which are generally equivalent to the MFI readings, described in Example 3a above, for comparative purposes) were quantified for each sample analyzed by flow cytometry. Background MedFI was subtracted from all data points. Percent inhibition was reported by calculating the percent

value of binding of full length human VWF to platelets in the presence of aptamer at a given concentration relative to binding in the absence of any aptamer (see Figure 7). IC_{50} values were determined by fitting the percent inhibition of vWF binding to platelets as a function of aptamer concentration to the equation:

$$\% \text{ inhibition} = \% \text{ inhibition}_{\max} / (1 + IC_{50} / \text{aptamer conc.})$$

Results of botrocetin induced rabbit A1 vWF binding characterization are tabulated below in Table 26.

Table 26: Results of FACS and BIPA Assays ('ND' = not done)

Aptamer ID	Inhibition of full length hVWF binding in FACS assay		Inhibition of rabbit VWF A1 domain binding in FACS assay		≤ 200 nM aptamer gives >90% blocking in BIPA at 6 minutes
	IC_{50} range	Average IC_{50}	IC_{50} range	Average IC_{50}	
rRdY aptamer aptamers					
(AMX203.D6) SEQ ID NO 31	no activity		ND		ND
(AMX205.H8) SEQ ID NO 32	no activity		ND		ND
(AMX205.H11) SEQ ID NO 33	no activity		ND		ND
(AMX205.D11) SEQ ID NO 35	no activity		ND		ND
(AMX206.F9) SEQ ID NO 36	no activity		ND		ND
(AMX206.H9) SEQ ID NO 37	no activity		ND		ND
(AMX206.A10) SEQ ID NO 38	no activity		ND		ND
(AMX205.F9) SEQ ID NO 39	no activity		ND		ND
(AMX206.E7) SEQ ID NO 40	no activity		ND		ND

(AMX206.D7) SEQ ID NO 41	no activity		ND		ND
(AMX203.A1) SEQ ID NO 43	no activity		ND		ND
(AMX203.G9) SEQ ID NO 44 = ARC 842	1.2nM to 8.5nM	4.7nM	152pM to 1.6nM	592pM	yes
(AMX205.H9) SEQ ID NO 45	no activity		ND		ND
(AMX206.D8) SEQ ID NO 46	no activity		no activity		ND
(AMX205.F7) SEQ ID NO 49 = ARC 841	321pM to 1.6nM	818pM	1.5nM to 7.1nM	4.7nM	yes
(AMX205.H10) SEQ ID NO 50	no activity		no activity		ND
rRdY aptamer minimers					
SEQ ID NO 177	1.6nM to 5.4nM	3nM	ND		ND
SEQ ID NO 180	731pM to 3.8nM	2nM	8.2nM to 10.5nM	9.3nM	yes
SEQ ID NO 183	6.6nM to 99nM	26.5nM	ND		ND
SEQ ID NO 186	971pM to 2.2nM	1.3nM	ND		ND
SEQ ID NO 189	564pM to 1.6nM	1.1nM	ND		ND
SEQ ID NO 192	1.1nM to 5.3nM	2.6nM	2nM to 2.3nM	2.2nM	yes
SEQ ID NO 194	no activity		ND		ND
SEQ ID NO 196	no activity		ND		ND
SEQ ID NO 198	14nM to 25nM	20.6nM	8.5nM to 16.8nM	12.6nM	ND
SEQ ID NO 201	1.3nM to 370nM	150nM	ND		no activity
rRfY aptamer aptamers					
(AMX201.B1) SEQ ID NO 11	no activity		ND		yes
(AMX198.G1) SEQ ID NO 12	no activity		ND		ND
(AMX201.H3) SEQ ID NO 13	no activity		ND		yes
(AMX201.G1) SEQ ID NO 15	no activity		ND		ND
(AMX201.C8) SEQ ID NO 23 = ARC 840	24pM to 1.8nM	528pM	141pM to 704pM	682pM	yes
rRfY aptamer minimers					
SEQ ID NO 165	562pM to 14.4nM	6.4nM	ND		ND
SEQ ID NO 169	103pM to 17nM	6.9nM	ND		ND
SEQ ID NO 172	1.8nM to 7.4nM	4.3nM	ND		ND
SEQ ID NO 174	1.3nM to 12.7nM	6.6 nM	4.9nM to	6.8nM	yes

			5nM		
DNA SELEX 1, minimizer					
ARC845 SEQ ID NO 205	no activity	no activity	no activity	no activity	no activity
DNA SELEX 2 aptamer aptamers					
AMX237.E10 SEQ ID NO 138	no activity		ND		ND
AMX237.G7 SEQ ID NO 134	2.3nM to 14nM	6.5nM	12.3nM		ND
AMX.236.G1 SEQ ID NO 164	8.6nM to 72nM	46nM			ND
AMX237.A11 SEQ ID NO 98	466pM to 5.6nM	2.2nM	4.7nM		ND
AMX237.A2 SEQ ID NO 99	815pM to 7.3nM	3.5nM	7nM		ND
AMX238.D12 SEQ ID NO 100	684pM to 3.4nM	2nM	7.2nM		ND
AMX238.G5 SEQ ID NO 106	273pM to 2.6nM	1.1nM	5.5nM		ND
AMX238.E9 SEQ ID NO 115	772pM to 6.5nM	3.4nM	ND		ND
AMX238.H5 SEQ ID NO 118	514pM to 860pM	658pM	ND		ND
AMX237.G6 SEQ ID NO 114	1.5nM to 2.5nM	1.9nM	ND		yes
AMX237.B11 SEQ ID NO 109	151pM to 4.8nM	2.5nM	5.7nM		ND
AMX236.A12 SEQ ID NO 127	1.2nM to 17nM	10.7nM	14.5nM		ND
DNA SELEX 2 aptamer minimizers					
SEQ ID NO 208	1.6nM to 10.2nM	4.3nM	ND		ND
SEQ ID NO 209	no activity		ND		ND
SEQ ID NO 210	no activity		ND		ND
SEQ ID NO 211	no activity		ND		ND
ARC1027 SEQ ID NO 212	208pM to 4.2nM	1.5nM	462pM to 4.2nM	2nM	yes
ARC1028 SEQ ID NO 213	473pM to 2.7nM	1.2nM	526pM to 2.7nM	1.5nM	yes
ARC1029 SEQ ID NO 214	333pM to 1.1nM	609pM	490pM to 979pM	754pM	yes
ARC1030 SEQ ID NO 215	no activity		ND		ND
ARC1031	no activity		ND		ND

SEQ ID NO 216					
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EXAMPLE 3C: Inhibition of Botrocetin induced platelet aggregation (BIPA assay)

[00329] In order to determine the activity of aptamers on live human platelets, BIPA assays were done using freshly prepared platelet rich plasma. Blood was obtained from healthy human donors who had not taken NSAIDS for at least five days. 21 ¾ gauge butterfly needles (Cat# 367287) from Becton Dickinson were used to draw blood into 0.105 M sodium citrate vacutainer tubes. Collected blood was pooled into 15 mL conical tubes and was spun at 200 g for 20 minutes. The turbid, yellow layer of platelet rich plasma ("PRP") was withdrawn from the tubes, pooled, and set aside at room temperature. The remaining blood was spun at 2500 g for ten minutes. The clarified layer of plasma, known as platelet poor plasma ("PPP"), was withdrawn and set aside at room temperature. PRP was aliquoted into cuvettes containing stir bars at a volume of 470 uL. A sample of 500 uL of PPP was aliquoted into a cuvette and placed in the PPP reference cell of the platelet aggregometer. Samples of PRP were prewarmed at 37 degrees C in the platelet aggregometer for 3-5 minutes before being used in BIPA assays. First, the concentration of botrocetin needed to induce platelet aggregation for each individual donor was determined by titration. This concentration of botrocetin was used for the remainder of the experiment. Next, aptamer was assayed by adding titrations of aptamer (zero, 1 nM to 1000 nM) to prewarmed PRP for one minute, followed by addition of botrocetin. With platelet aggregation, an increase in amplitude of light transmission is seen (Figure 8). The concentration at which platelet aggregation is blocked at 90% or greater after 6 minutes is reported in the final column of Table 26. Using the Aggro/LINK Software, area under the curve ("AUC") can be generated from the aggregometer trace and used to calculate percent inhibition of aptamer at any given concentration on botrocetin induced platelet aggregation as seen in Figure 9 for Aptamer ARC1029 (SEQ ID NO 214).

EXAMPLE 3D: Biological activity of selected modified aptamers in a series of biological assays:

[00330] ARC1172 (SEQ ID NO 222), ARC1346 (SEQ ID NO 281), ARC1368 (SEQ ID NO 291), ARC1525 (negative control), ARC1779 (SEQ ID NO 320), ARC1780 (SEQ ID

NO 321), and ARC1885 (SEQ ID NO 323) identified in the medchem modification process described in Example 2 above, were tested in a series of biological assays. These assays included the FACS (as described in Example 3A and 3B) and BIPA assays (as described in Example 3C), as well as the platelet PFA-100 assay described below.

Materials:

[00331] The following materials were used in the platelet function analyzer (PFA) assay: Fresh whole blood was collected from healthy non-steroidal anti-inflammatory drug (NSAID) free donors into 5ml 0.105M sodium citrate tubes (Cat#369714, Becton Dickinson) using 21¼ gauge butterfly needles (Cat#367287, Becton Dickinson). Fresh whole blood was collected from healthy non-steroidal anti-inflammatory drug (NSAID) free cynomolgus macaques. Aptamers were diluted with physiological saline from Aldon (Cat#9420306) in no-additive vacutainer tubes (Cat#366434, Becton Dickinson). Samples were loaded onto collagen/epinephrine test cartridges (Cat#B4170-20A, Dade Behring) which were used in the PFA-100 machine (Dade Behring). Trigger solution (Cat#B4170-50, Dade Behring) was used in the self-test and to pre-wet the test cartridges. O-ring cleaning pads (Cat#B4170-73, Dade Behring) were used in the self-test and in the O-ring cleaning process.

PFA assay:

[00332] A self-test, which included an O-ring cleaning process, was always run on the PFA-100 machine before running any samples to ensure proper function of the machine.

[00333] Fresh whole blood was collected from healthy donors or cynomolgus macaques, as indicated in Table 27 below, who/that had not taken NSAIDs for at least three days. Blood from human donors was collected into 5ml 0.105M sodium citrate tubes using a 21¼ gauge butterfly needle, and the tubes were gently inverted three times to ensure mixture of blood with sodium citrate. During the entire experiment, the tubes of whole blood were gently inverted every five minutes to prevent settling.

[00334] In order to assay the titration of aptamer in whole blood, the aptamer was added to no-additive vacutainer tubes and diluted to the desired concentrations (ex: 0nM, 1nM to 1000nM) using physiological saline such that the final volume was 60µl. When the PFA-100

was ready to run the next set of samples, 1940µl of whole blood was added to the tube containing a concentration of aptamer. This tube was gently inverted three times to thoroughly mix the aptamer and blood. Samples were always run in duplicate on the PFA-100 machine. 800µl of this blood mixture was loaded into the collagen/epinephrine test cartridges. The test cartridges were loaded onto the PFA-100 machine. The time of occlusion of the aperture was measured by the PFA-100, with a maximal time of 300seconds. We estimate the IC₉₅ in this assay to be the minimum concentration of aptamer that extends the closing time to 300 seconds.

[00335] Figure 20 depicts clotting time in human whole blood as a function of aptamer concentration in the PFA-100 assay for ARC1368 (SEQ ID NO 291) and the negative control ARC1525. Additional results of the FACS, BIPA and PFA-100 assays are tabulated in Table 27 below.

Table 27: FACS, BIPA and PFA-100 results

ARC #	FACS IC ₅₀ vs human full length vWF (nM)	FACS IC ₅₀ vs rabbit A1 domain (nM)	~IC ₉₀ BIPA (nM) with human platelet rich plasma	~IC ₉₅ PFA-100 (nM) with citratd human whole blood	~IC ₉₅ PFA-100 (nM) with citratd C. macaque whole blood
ARC1172 (SEQ ID NO 222)	2	2	~200	~100	ND
ARC1346	50	180	> 1000	ND	ND
ARC1368	2	4.0	~200	~100	~100
ARC1525	ND	ND	no inhibition	no inhibition	ND
ARC1779	ND	ND	~100	~100	ND
ARC1780	ND	ND	~100	~100	ND
ARC1885	ND	ND	~50	~100	ND

[00336] As expected, there was a strong correlation among the observed affinities of aptamers for vWF in the binding assay described in Example 2 above and their relative potency in the biological assays. The non-binding negative control ARC1525 did not display activity in any assay in which it was tested

EXAMPLE 3E: ARC1368, Integrilin™ and ReoPro™ in BIPA, PFA-100 and AIPA assays

[00337] The potency of ARC1368 (SEQ ID NO 291), Integrilin™ and ReoPro™ were evaluated in human whole blood in PFA-100, in human PRP in BIPA (as described above in Examples 3D and 3C respectively) and ADP Induced Platelet Aggregation (AIPA) assays. AIPA was performed with human PRP exactly as was done for BIPA described above in Example 3C with the exception that instead of adding botrocetin, 10 micromolar ADP (Chronolog, Haverton, PA) was added to induce platelet aggregation. The PFA-100 results are shown in Figure 21. The BIPA results are shown in Figure 22. The AIPA results are shown in Figure 23. As can be seen in Figures 21 and 22, ARC1368 (SEQ ID NO 291) shows potency

comparable to ReoPro™ in PFA-100 and BIPA assays. Consistent with the vWF dependent mechanism described above, the anti-vWF aptamer shows no ability to block AIPA while the IIbIIIa antagonists remain potent in that assay as shown in Figure 23.

EXAMPLE 4: PHARMACOKINETIC STUDIES

[00338] In Examples 4 and 5, all mass based concentration data refers only to the molecular weight of the oligonucleotide portion of the aptamer, irrespective of the mass conferred by PEG conjugation.

EXAMPLE 4A: Stability of anti-vWF aptamers in human and rat plasma

[00339] ARC1172 (SEQ ID NO 222), ARC1346 (SEQ ID NO 281), ARC1368 (SEQ ID NO 291) and ARC1533 were assayed for nuclease stability in both human and rat plasma. Plasma nuclease degradation was measured on denaturing polyacrylamide gel electrophoresis as described below. Briefly, for plasma stability determination, chemically synthesized aptamers were purified using denaturing polyacrylamide gel electrophoresis, 5' end labeled with γ -³²P ATP and then gel purified again. Trace 32-P labeled aptamer was incubated in the presence of 100 nM unlabeled aptamer in 95% human or rat plasma in a 200 microliter binding reaction. The reaction for the time zero point was made separately with the same components except that the plasma was replaced with PBS. This insured that the amount or radioactivity loaded on gels was consistent across an experiment. Reactions were incubated at 37 °C in a thermocycler for the 1, 3, 10, 30 and 100 hours unless otherwise specified. At each time point, 20 microliters of the reaction was removed, combined with 200 microliters of formamide loading dye and flash frozen in liquid nitrogen and stored at -20 °C. After the last time point was taken, frozen samples were thawed and 20 microliters was removed from each time point. SDS was then added to the small samples to a final concentration of 0.1%. The samples were then incubated at 90 °C for 10 – 15 minutes and loaded directly onto a 15% denaturing PAGE gel and run at 12 W for 35 minutes. Radioactivity on the gels was quantified using a Storm 860 phosphorimager system. The percentage of full length aptamer at each time point was determined by quantifying the full length aptamer band and dividing by the total counts in the lane. The fraction of full length aptamer at each time-point was then normalized to the percentage full length aptamer of the 0 hour time-point. The fraction of full length aptamer as a function of time was fit to the equation:

[00340] $m_1 * e^{(-m_2 * m_0)}$

where m_1 is the maximum % full length aptamer ($m_1=100$); and m_2 is the rate of degradation. The half-life of the aptamer ($T_{1/2}$) is equal to the $(\ln 2) / m_2$.

[00341] Sample data for human plasma is shown in Figure 24 and the results for the aptamers tested are summarized in Table 28. Consistent with our expectations, aptamers are more stable in human plasma than in rat plasma and increasing the number of 2'-OMe modifications correlates with increasing plasma stability.

Table 28: Aptamer Plasma Stability half-life

ARC #	T1/2 Human plasma (hrs)	T1/2 Rat plasma (hrs)
ARC1172 (SEQ ID NO 222)	17	3
ARC1346	not done	19
ARC1368	63	21
ARC1533	93	not done

EXAMPLE 4B: PK/PD of PEGylated derivatives of ARC1368 in Cynomolgus Macaques

[00342] ARC1368 (SEQ ID NO 291), 1779 (SEQ ID NO 320) and 1780 (SEQ ID NO 321) (as described in Example 2 above) were injected intravenously into cynomolgus macaques (n = 3 / group) at a dosage of 3 mg/kg which was expected to yield an instantaneous plasma concentration of 3 uM, approximately 30-fold higher than the putative

effective dose. Subsequently, citrated blood samples were collected at regular intervals and processed for plasma.

[00343] To demonstrate that the aptamers were pharmacologically active *in vivo*, Botracetin-induced platelet aggregation (BIPA) was performed 5 minutes post-dosing, at the presumed plasma C_{max} . All animals had complete inhibition of BIPA at this point demonstrating that the aptamers were functional *in vivo*.

[00344] Subsequently, plasma aptamer concentrations were determined using the Oligreen assay (Gray *et al.*, Antisense and Nucleic Acid Drug Development 7 (3):133-140 (1997) . The data were subsequently analyzed using the program WinNonlin to yield the pharmacokinetic parameters listed in Tables 29 to 31 below.

[00345] Additionally, the primate plasma aptamer concentration plotted as a function of time is depicted in the graph of Figure 25. The mean concentration-times profiles based on the OliGreen™ assay showed that the pharmacokinetic profiles of ARC1368 (SEQ ID NO 291), ARC1780 (SEQ ID NO 321) and ARC1779 (SEQ ID NO 320) were mainly monophasic. The unPEGylated aptamer (ARC1368 (SEQ ID NO 291)) displayed a rapid distribution phase compared to ARC1779 (SEQ ID NO 320) and ARC1780 (SEQ ID NO 321). Unlike ARC1368 (SEQ ID NO 291), the 40 kDa PEG conjugate ARC 1780 (SEQ ID NO 321) displayed prolonged distribution phase compared to ARC1779 (SEQ ID NO 320). ARC1779 (20 kDa PEG) displayed a distribution phase with α -half-life of ~2 hr.

Table 29 - NonCompartmental Pharmacokinetic Parameter Estimates for ARC1368 After 3 mg/kg IV Administration in Monkeys Based on Oligreen Assay Data

PK Parameter	Unit	1101	1102	1103	Mean	StdDev
Tmax	hr	0.08	0.08	0.08	0.08	0.00
Cmax	ng/mL	26409	19170	23301	22960	3632
AUC0-last	hr*ng/mL	23096	31926	20650	25224	5932
MRTlast	hr	4.50	5.46	3.48	4.48	0.99

Table 30 - NonCompartmental Pharmacokinetic Parameter Estimates for ARC1779 After 3 mg/kg IV Administration in Monkeys Based on Oligreen Assay Data

PK Parameter	Unit	2101	2103	2104	Mean	StdDev
Tmax	hr	0.25	0.50	0.25	0.33	0.14
Cmax	ng/mL	69065	65717	70344	68375	2389
AUClast	hr*ng/mL	309245	336590	235503	293779	52288

MRTlast	hr	5.27	4.66	2.69	4.21	1.35
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Table 31 - NonCompartmental Pharmacokinetic Parameter Estimates for ARC1780 After 3 mg/kg IV Administration in Monkeys Based on Oligreen Assay Data

PK Parameter	Unit	3101	3102	3103	Mean	StdDev
Tmax	hr	2.00	2.00	0.25	1.42	1.01
Cmax	ng/mL	55965	37320	50690	47992	9611
AUClast	hr*ng/mL	740559	613180	899455	751065	143426
AUCall	hr*ng/mL	740559	613180	899455	751065	143426
MRTlast	hr	8.69	9.54	14.11	10.78	2.91

[00346] Following the Oligreen assay analysis, plasma aptamer concentrations were determined for the animals dosed with ARC1779 (SEQ ID NO 320) using a validated HPLC-based assay. The HPLC data were analyzed via noncompartmental and 2-compartment analysis using the program WinNonlin. Reanalysis of the monkey samples with a more sensitive HPLC method generated a concentration-times profile of ARC1779 (SEQ ID NO 320) to be biphasic showing both distribution and elimination phase. Consistent with the results observed using the OliGreen assay, the HPLC-based results indicated the distribution half-life ($t_{1/2\alpha}$) was 1.4 h and a elimination half-life was 12.9 ($t_{1/2\beta}$) for ARC1779 (SEQ ID NO 320).

EXAMPLE 4C: PK/PD of ARC1779 in Cynomolgus Macaques

[00347] -PK/PD correlation of ARC1779 was evaluated in three cynomolgus macaques after a single intravenous (IV) bolus dose at 0.5 mg/kg. ARC1779 levels in the plasma were correlated to PD effects of ARC1779 in inhibition of platelet function or prolongation of cutaneous bleeding time (CBT).

[00348] Following IV administration, blood was collected percutaneously at various time points post-dose for PK and PD analysis. PD effect of ARC1779 on platelet function was determined by PFA-100 assays and effect of ARC1779 on bleeding was measured by CBT time. In addition, plasma samples were analyzed by HPLC for quantitation of ARC1779 levels. PK parameter estimates were determined by 2-compartment analysis. The results are presented in Figure 26.

[00349] The concentration-time profiles generated for individual monkeys showed predominantly the distribution phase of ARC1779 pharmacokinetics. The distribution half-life was determined to be approximately 1.0 hour ($t_{1/2\alpha}$). The elimination half-life ($t_{1/2\beta}$), was not well-determined from the available data.

[00350] The PD effect of ARC1779 on platelet aggregation measured by the PFA-100 assay is shown in Figure 26. When the plasma concentrations of ARC1779 were in excess of 300 nM, platelet function was inhibited as assessed by the PFA-100 instrument. However, when plasma aptamer concentrations decreased to approximately 77 nM, platelet function returned to normal.

[00351] As also seen in Figure 26, the PD effect of ARC1779 on bleeding time prolongation was found to be minimal in these studies.

[00352] In summary, ARC1779 inhibited platelet function *in vivo* at a plasma concentration of approximately 300 nM. This *in vivo* concentration was approximately 3-fold higher than the observed concentration of aptamer necessary to inhibit platelet function *in vitro*. In contrast, even at high plasma concentrations (1000 nM), ARC1779 showed minimal effect on cutaneous bleeding time following single bolus dosing.

EXAMPLE 5: FUNCTIONAL ANIMAL ASSAYS

[00353] In Examples 4 and 5 described herein, all mass based concentration data refers only to the molecular weight of the oligonucleotide portion of the aptamer, irrespective of the mass conferred by PEG conjugation.

EXAMPLE 5A: Pharmacodynamics of ARC1779 in Cynomolgus Macaques

[00354] C. macaques were dosed at 0.5 mg/kg IV bolus with ARC1779 (SEQ ID NO 320). PFA-100 closure time, BIPA and cutaneous bleeding time ("CBT") were all measured as a function of time throughout the studies. BIPA and PFA-100 closure times were measured as described in previously in Example 3. Cutaneous bleeding times were measured using standard protocols described as follows.

[00355] A blood pressure cuff was applied to the biceps region of the forearm to be tested and inflated to maintain a constant pressure of 40 mmHg. Using a Surgicutt® Automated Incision Device (ITC, Edison, NJ), a longitudinal incision was made over the lateral aspect of the volar surface of the forearm distal to the antecubital crease. A stopwatch was started at the time of incision. At 15-30 seconds post incision, the blood was wicked with Surgicutt® Bleeding Time Blotting Paper (ITC, Edison, NJ) while avoiding direct contact with the incision. Every 15-30 seconds the blotting paper was rotated and re-blotted at a fresh site on the paper. The blotting was repeated until blood was no longer wicked onto the paper or for 30 minutes whichever came first. Bleeding time is determined to within 30 seconds of the time when blood is no longer wicked onto the paper.

[00356] The table of Figure 27 shows the cutaneous bleeding time in minutes, BIPA IC₉₀ in nM and PFA IC₉₅ in nM at various time points, shown in column 1, relative to ARC1779 (SEQ ID NO 320) dosing for three different animals. Figure 28 shows a graph of the average PFA-100 closure time from the blood of three ARC1779 (SEQ ID NO 320) treated macaques taken at various time points following dosing. Figure 29 shows the cutaneous bleeding time of the three ARC1779 (SEQ ID NO 320) treated macaques taken at various time points following dosing. Figure 30 correlates the average cutaneous bleeding time in ARC1779 (SEQ ID NO 320) treated C. macaques (left vertical axis) to the PFA-100 closure time (right vertical axis). As shown Figures 27 to 30, at time points up to and including 2 hours, where BIPA and PFA-100 closure time were maximally inhibited, there is very little increase in cutaneous bleeding times. At concentrations of the anti-gpIIb/IIIa antagonist Integrilin™ that yield similar inhibition of platelet aggregation *ex vivo*, template or cutaneous bleeding times are between 20 and 30 minutes. See, e.g. Phillips, D.R. and Scarborough, R.M., Am J Cardiol, 80(4A):11B-20B (1997). While not wishing to be bound by any particular theory, these data are consistent with and supportive of our hypothesis that an anti-vWF A1 domain aptamer antagonist will block platelet activity *in vivo* by blocking platelets from binding to vWF immobilized at sites of vascular damage without inhibiting platelet function and thus without increasing cutaneous bleeding times.

EXAMPLE 5B: Assessment of ARC1779 in a cynomolgus macaque electrolytic thrombosis model

[00357] A study was performed to test the efficacy of ARC1779 (SEQ ID NO 320) in inhibiting intra-arterial thrombosis in a well documented non-human primate electrolytic thrombosis model. See, *e.g.*, Rote *et al.*, Stroke, 1994: 25, 1223-1233. Thirteen cynomolgus monkeys were divided into four groups and assigned to a treatment regimen as indicated in table 30 below.

Table 32: Electrolytic Thrombosis Study Design

Group Number	Number of Animals	Test Article	Dose (mg/kg)	Dose Regime	Dose Volume	Necropsy Day
1	3	Vehicle (saline)	DVE to Group 3	IV bolus, ~15 minutes prior to initiation of electrical injury followed by continuous infusion on Day 0	≤ 10mL	Day 0
2	1	ReoPro™ (chimeric 7E3)	0.25 mg/kg	IV bolus, once on Day 0, ~15 minutes prior to initiation of electrical injury		
3	5	ARC1779	0.61 mg/kg bolus + 0.0037 mg/kg/min infusion	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0		
4	4	ReoPro™ (chimeric 7E3)	0.25 mg/kg bolus + 0.125 µg/kg/min infusion	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0		

DVE = Dose Volume Equivalent, IV = Intravenous

[00358] Each animal was anesthetized prior to surgical preparation, intubated and maintained in anesthesia with isoflurane inhalant anesthetic to effect delivered through a volume-regulated respirator. An intravenous catheter was also placed in a peripheral vein for administration of lactated Ringer's solution during the procedure.

[00359] A catheter was placed in the femoral artery of each animal for continuous monitoring of arterial blood pressure. Similarly a catheter was placed in the femoral vein for blood sample collection. Each carotid artery was instrumented with a Doppler flow probe connected to a flow meter. The flow probes were placed around the artery at a point proximal

to the insertion of the intra-arterial electrode and stenosis. The stenosis was placed around each carotid artery so that the blood flow was reduced by approximately 50% to 60% without altering the mean blood flow. Blood flow in the carotid arteries was monitored and recorded continuously throughout the observation periods.

[00360] Electrolytic injury to the intimal surface of each carotid artery was accomplished via placement of an intravascular electrode. Each electrode was connected to the positive pole of a constant current device and cathode connected to a distant subcutaneous site. Continuous current was delivered to each vessel for a period of 3 hours or for 30 minutes after complete occlusion, whichever was shorter.

[00361] Once the electrodes were placed on the right carotid artery ("RCA") the animal was administered the test article as indicated in Table 32 above. Approximately 15 minutes after test article administration, the electrical current was applied at 100 μ A. Blood samples and CBT measurements were collected at the time points specified in Blood Sample Collection Schedule as indicated in Figure 31. The current was terminated ~30 minutes after the blood flow signal remained stable at zero flow (which indicates an occlusive thrombus had been formed at the site) or after 180 minutes of electrical stimulation. Approximately 195 minutes after the test article was administered, the left carotid artery ("LCA") had electrical current administered in a similar fashion as previously described for the RCA. After termination of all surgical procedures and sample collection each animal was euthanized.

[00362] The ARC1779 (SEQ ID NO 320) plasma concentration (as measured by HPLC) over time for each animal in treatment group 3 is depicted in Figure 32. The time to occlusion measured via Doppler flow for each treatment group is indicated in Figure 33. As can be determined from Figure 33, ARC1779 (SEQ ID NO 320) inhibited thrombus formation during sequential 180-minute electrical injuries to the carotid arteries of the cynomolgus monkeys in this animal thrombosis model.

Example 5C: Assessment of ARC1779 at various doses in a cynomolgus macaque electrolytic thrombosis model

[00363] The study described in Example 5B above was extended to test the efficacy of ARC1779 (SEQ ID NO 320) in inhibiting intra-arterial thrombosis in the non-human primate electrolytic thrombosis model at lower aptamer dosage levels..

[00364] An additional 10 cynomolgus monkeys (2.5 to 3.5 kg) were divided into treatment groups 5 to 7 and were treated according the regimen indicated in Table 33 below.

Table 33: Study Design

Group Number	No. of Animals	Test Article	Dosage Level	Dose Volume	Dosing Regimen	Necropsy Day
1	3	Vehicle (Saline)	DVE to Group 3	≤10 mL	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0	Day 0
2	1	Abciximab (ReoPro)	0.25 mg/kg		IV bolus, once on Day 0 ~15 minutes prior to initiation of electrical injury	
4	5	Abciximab (ReoPro)	0.25 mg/kg bolus and 0.125 µg/kg/min		IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0	
3	5	ARC1779	0.61 mg/kg bolus and 0.0037 mg/kg/min infusion (1000 nM)	≤10 mL	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0	Day 0
5	2	ARC1779	0.123 mg/kg bolus and 0.001 mg/kg/min infusion (300 nM)	≤10 mL	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0	Day 0
6	4	ARC1779	0.2 mg/kg bolus and 0.00165 mg/kg/min infusion (500 nM)	≤10 mL	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0	Day 0
7	4	ARC1779	0.298 mg/kg bolus and 0.00248 mg/kg/min infusion (750 nM)	≤10 mL	IV bolus, ~15 minutes prior to initiation of electrical injury, followed by continuous infusion on Day 0	Day 0

[00365] The animal procedure for each treatment group in Table 33 was conducted as reported for the animals in Example 5B. The time to occlusion measured via Doppler flow for each treatment group is indicated in Figure 33.

[00366] Without any platelet antagonist therapy (control animals) 100% of carotid arteries develop occlusive thrombi within 60 minutes. In contrast, only 20% of arteries developed occlusive thrombi in animals treated with Reopro. In the ARC1779 treatment groups at infusion rates targeted to reach constant plasma levels of 1000 nM, 750 nM, 500 nM and 300 nM, 0%, 25%, 63% and 100 % of carotid arteries developed occlusive thrombi. In addition to occlusive thrombus formation, we also assessed the effect of ARC1779 on cutaneous bleeding

times which is shown in Figure 34. As can be seen in Figure 34, at some doses and/or time points in this animal model prolonged CBT was observed, while at other doses and time points CBT was not prolonged.

[00367] The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the description and examples above are for purposes of illustration and not limitation of the following claims.

What is claimed is:

- 1) An aptamer comprising a nucleic acid sequence that is 95% identical to a sequence selected from the group consisting of: ARC1029 (SEQ ID NO 214), ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284), ARC1368 (SEQ ID NO 291), SEQ ID NO 1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), and ARC1884 (SEQ ID NO 322).
- 2) The aptamer of claim 1, wherein the aptamer comprises a primary nucleic acid sequence that is 95% identical to a primary nucleic acid sequence selected from the group consisting of: ARC1029 (SEQ ID NO 214), ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284), ARC1368 (SEQ ID NO 291), SEQ ID NO 1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), and ARC1884 (SEQ ID NO 322).
- 3) The aptamer of claim 1, wherein the aptamer nucleic acid sequence comprises a chemical modification and is 95% identical to a nucleic acid sequence, including the chemical modification, selected from the group consisting of: ARC1029 (SEQ ID NO 214), ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284), ARC1368 (SEQ ID NO 291), SEQ ID NO 1635 (SEQ ID NO 319), ARC1759 (SEQ ID NO 318), and ARC1884 (SEQ ID NO 322).
- 4) The aptamer of claim 1, wherein the aptamer is conjugated to a high molecular weight, non-immunogenic compound, or a lipophilic compound.
- 5) The aptamer of claim 4, wherein the aptamer is conjugated to a non-immunogenic, high molecular weight compound which is polyalkylene glycol.
- 6) The aptamer of claim 5, wherein the polyalkylene glycol is polyethylene glycol.
- 7) The aptamer of claim 6, wherein the polyethylene glycol comprises a molecular weight selected from the group consisting of: 5kDa, 10 kDa, 20kDa and 40 kDa.
- 8) The aptamer of claim 7, wherein the aptamer is selected from the group consisting of: ARC1779 (SEQ ID NO 320), ARC1780 (SEQ ID NO 321) and ARC1885 (SEQ ID NO 323).

- 9) A composition comprising a therapeutically effective amount of an aptamer of claim 1 or a salt thereof and a pharmaceutically acceptable carrier or diluent.
- 10) A method of treating, preventing or ameliorating a disease mediated by vWF, comprising administering the composition of claim 9 to a mammal.
- 11) A method of treating a patient comprising administering the composition of claim 9 to a patient in need thereof prior to, during and/or after dialysis, CABG surgery, percutaneous coronary intervention or heart valve replacement.
- 12) The method of claim 10, wherein the composition comprises ARC1368 (SEQ ID NO 291) or a fragment thereof conjugated to a 20 kDA PEG.
- 13) The method of claim 12, wherein the composition comprises ARC1779 (SEQ ID NO 320)
- 14) The method of claim 11, wherein the composition comprises ARC1368 (SEQ ID NO 291) or a fragment thereof conjugated to a 20 kDA PEG.
- 15) The method of claim 14, wherein the composition comprises ARC1779 (SEQ ID NO 320).
- 16) A composition comprising therapeutically effective amount of an aptamer of claim 8 or a salt thereof and a pharmaceutically acceptable carrier or diluent.
- 17) A method of treating, preventing or ameliorating a disease mediated by vWF, comprising administering the composition of claim 16 to a mammal.
- 18) An aptamer that specifically binds to a von Willebrand Factor target.
- 19) The aptamer of claim 18, wherein the von Willebrand Factor target is human von Willebrand Factor.
- 20) The aptamer of claim 18, wherein the aptamer modulates von Willebrand Factor mediated platelet aggregation.
- 21) An aptamer according to claim 18 that specifically binds to a von Willebrand Factor full length target and a von Willebrand Factor domain A1 target.

22) The aptamer of claim 21, wherein the von Willebrand Factor full length target and the von Willebrand Factor domain A1 target are from different species.

23) A diagnostic method comprising contacting an aptamer of any of claims 18 with a composition suspected of comprising von Willebrand Factor or a variant thereof and detecting the presence or absence of von Willebrand Factor or a variant thereof.

24) A method for identifying an aptamer that modulates a biological function of an aptamer target comprising:

- a) preparing a candidate mixture of single-stranded nucleic acids;
- b) contacting the candidate mixture with both a full length protein target and a domain of the full length protein target;
- c) partitioning the nucleic acids having an increased affinity for the full length protein target or the protein target domain; and
- d) amplifying the increased affinity nucleic acids, *in vitro*, to yield a protein target specific enriched aptamer mixture.

25) The method of claim 24, wherein the method further comprises;

- e) contacting the target specific enriched aptamer mixture with the full length protein target;
- f) partitioning the nucleic acids having an increased affinity for the full length protein target; and
- g) amplifying the increased affinity nucleic acids, *in vitro*; to yield a target specific enriched aptamer mixture;
- h) contacting the target specific enriched aptamer mixture with the protein target domain;
- f) partitioning the nucleic acids having an increased affinity for the protein target domain; and
- g) amplifying the increased affinity nucleic acids, *in vitro*, to yield a protein target specific enriched aptamer mixture.

- 26) The method of claim 25, wherein the method further comprises selecting an aptamer that blocks a biological function of the full length protein target *in vivo*.
- 27) The method of claim 25, wherein the full length protein target is from a first species and the protein target domain is from a second species.
- 28) The method of claim 27, further comprising selecting an aptamer capable of binding to the protein targets of both the first and second species.
- 29) An aptamer identified by the method of claim 26.
- 30) An aptamer that specifically binds to von Willebrand Factor comprising a primary nucleic acid sequence at least 95% identical to any one of the primary nucleic acid sequences selected from the group consisting of: SEQ ID NOS 31 to 50, SEQ ID NOS 54 to 94, SEQ ID NOS 98 to 164, SEQ ID NO 177, SEQ ID NO 180, SEQ ID NO 183, SEQ ID NO 186, SEQ ID NO 189, SEQ ID NO 192, SEQ ID NO 198, SEQ ID NO 201, SEQ ID NO 205, SEQ ID NO 208, SEQ ID NOS 212-214, ARC1115 (SEQ ID NO 221), ARC1172 (SEQ ID NO 222), ARC1194 (SEQ ID NO 223) to ARC1240 (SEQ ID NO 269), ARC1338 (SEQ ID NO 273) to ARC1346 (SEQ ID NO 281), ARC1361 (SEQ ID NO 284) to ARC1381 (SEQ ID NO 304), ARC1524 (SEQ ID NO 305), ARC1526 (SEQ ID NO 307) to ARC1535 (SEQ ID NO 316), ARC1546 (SEQ ID NO 317), ARC1759 (SEQ ID NO 318), ARC1635 (SEQ ID NO 319), ARC1779 (SEQ ID NO 320) to ARC1780 (SEQ ID NO 321) and ARC1884 (SEQ ID NO 322) to ARC1885 (SEQ ID NO 323).
- 31) A composition comprising therapeutically effective amount of an aptamer of claim 30 or a salt thereof and a pharmaceutically acceptable carrier or diluent.
- 32) A method of treating, preventing or ameliorating a disease mediated by vWF, comprising administering the composition of claim 31 to a mammal.
- 33) A method of treating a patient comprising administering the composition of claim 31 to a patient in need thereof prior to, during and/or after dialysis, CABG surgery, percutaneous coronary intervention or heart valve replacement.
- 34) A diagnostic method comprising contacting an aptamer of 30 with a composition suspected of comprising von Willebrand Factor or a variant thereof and detecting the presence or absence of von Willebrand Factor or a variant thereof.

- 35) An aptamer according to claim 30 for use as an *in vitro* diagnostic.
- 36) An aptamer according to claim 30 for use as an *in vivo* diagnostic.
- 37) The method of claim 24, wherein the aptamer target is von Willebrand Factor.
- 38) The method of claim 24, wherein the domain of the full length protein target is von Willebrand Factor domain A1.
- 39) The method of claim 25, wherein the full length protein target is von Willebrand Factor.
- 40) The method of claim 39, wherein the protein target domain is von Willebrand Factor domain A1.
- 41) The method of claim 28, wherein the protein target of both the first and second species is a von Willebrand Factor target.
- 42) An aptamer identified according to the method of claim 41.

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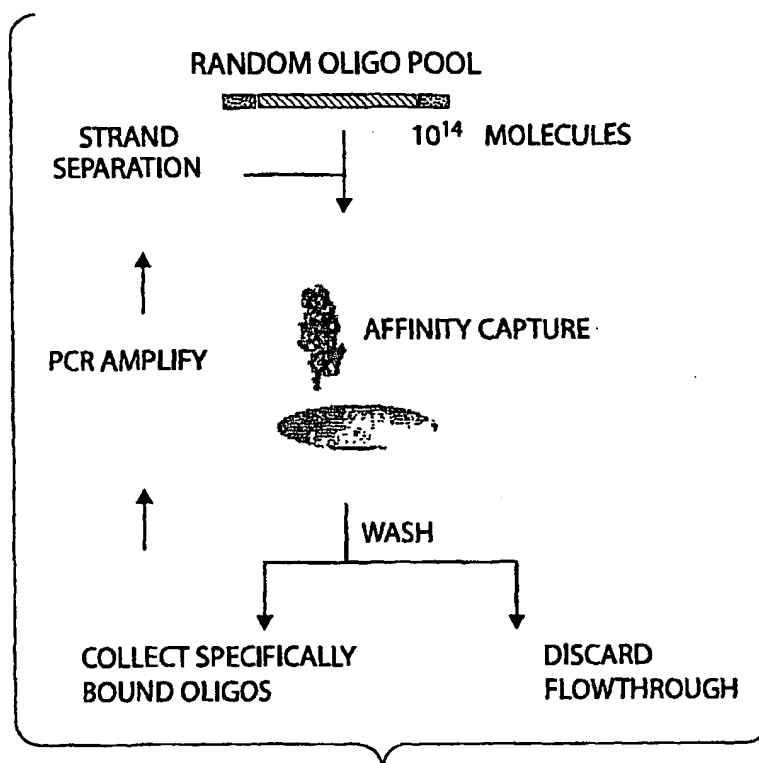


Fig. 1

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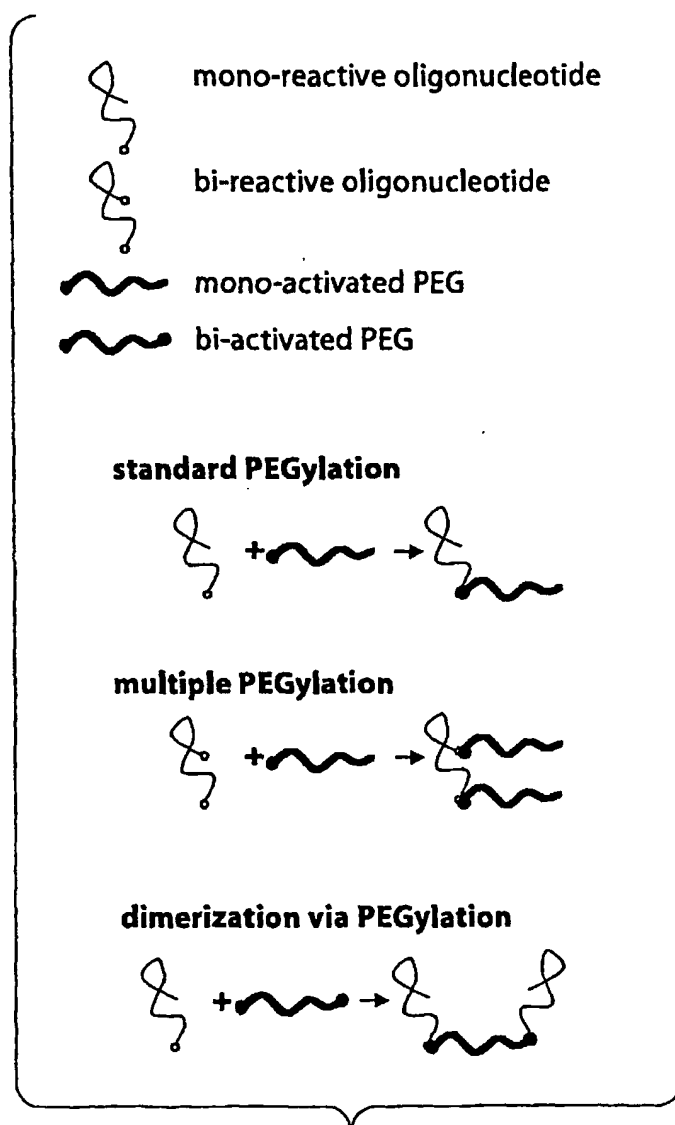


Fig. 2

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Amino Acid Sequence of Human vWF domain A1

MGHHHHHHEPPLHDFYCSRLDLVFLLDGSSRLSEAEFEVLKAFVVDMMEQLRISQK
WVRVAVVEYHDGSHAYIGLKDRKRPELRRIASQVKYAGSQVASTSEVLKYTLFQIFSK
IDRPEASRIALLMASQEPQRM SRNFVRYVQGLKKKKVIVIPVGIGPHANLKQIRLIEKQ
APENKAFVLSSVDELEQQRDEIVSYLCDLAPEAPPPT (SEQ. ID. No. 4)

Amino Acid Sequence of Human vWF domain A1

MRGSHHHHHHGSQEPGGLVVPPTDAPVSPTTLYVEDISEPPLHDFYCSRLDLVFLLD
GSSRLSEAEFEVLKAFVVDMMERLRISQKWVRVAVVEYHDGSHAYIGLKDRKRPELR
RIASQVKYAGSQVASTSEVLKYTLFQIFSKIDRPEASRIALLMASQEPQRM SRNFVRY
VQGLKKKKVIVIPVGIGPHANLKQIRLIEKQAPENKAFVLSSVDELEQQRDEIVSYLCDLA
PEAPPPTLPP (SEQ. ID. NO.5)

Amino Acid Sequence of Rabbit vWF domain A1

MGHHHHHHEPPLHDFYWSNLMDLVFLLDGSAQLSEAEFGVLKAFVVSVMERLHISQK
RIRVAVVEYHDGSHSYISLKDRKRPELRRIASQVKYAGGPVASTSEVLKYTLFHIFSNV
DRPEASRIALLSASQETPRMVRNLVRYAQGLKKEKVIVIPVGIGPHVSLRQIHLEKQA
PENKAFVLSGVDELEQRRDEIISYLCDLGPEAPVPT (SEQ. ID. NO. 6)

Fig. 3

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Amino Acid Sequence of Full Length Human vWF

MIPARFAGVLLALALILPGTLCAEGTRGRSSTARCSLFGSDFVNTFDGSMYSFAGYCSYLLAGG
 CQKRFSIIGDFQNGKRVSLSVYLGEFFDIHLFVNGTVTQGDQRVSMYPYASKGLYLETEAGYYK
 LSGEAYGFVARIDGSGNFOVLLSDRYFNKTCGLCGNFNIFAEDDFMTQEGTLTSDPYDFANSW
 ALSSGEQWCERASPPSSSCNISSGEMQKGLWEQCQLLKSTSVFARCHPLVDPEPFVALCEKT
 LCECAGGLECACPALLEYARTCAQEGMVLVYGTWDHSACSPVCPAGMEYRQCVSPCARTCQS
 LHINEMCQERCVDGCSCPEGQLLDEGLCVESRECPCVHSGKRYPPGTSLSRDCNTCICRNSQ
 WICSNEECPEGCLVTGQSHFKSFDNRYFTFSGICQYLLARDCQDHSFSIVIETVQCADDRDAVC
 TRSVTVRLPGLHNSLVKLKHGAGVAMDGQDIQLPLLKGDRLRIQHTVTASVRLSYGEDLQMDWD
 GRGRLLVKLSPVYAGKTCGLCGNYNGNQGDFTLPSGLAEPVEDFGNAWKHLHGDCQDLQK
 QHSDPCALNPRMTRFSEEACAVLTSPTFEACHRAVSPLPYLRNCRYDVCSCSDGRECLCGAL
 ASYAAACAGRGVRVAWREPGRCELNCPKGQVYLQCGTPCNLTCSRSLYPDEECNEACLEGC
 FCPPGLYMDERGDCVPKAQCPCYYDGEIFQPEDIFSDHHTMCYCEDGFMHCTMSGVPGSLLP
 DAVLSSPLSHRSKRSLSCRPPMVKLVCADNLRAGELECTKTCQNYDLECMMSGCVSGCLCP
 PGMVRHENRCVALERCPCFHQKEYAPGETVKIGCNTCVCRRDKWNCTDHVCDATCSTIGM
 AHYLTFDGLKYLFPGECCQYVLVQDYCGSNPGTFRILVGNKGCSHPSVKCKKRVTLVEGGEIEL
 FDGEVNVKRPMDKETHFEVVESGRYIILLGKALSVVWDRHLSISVVLKQTYQEKVCGLCGNFD
 GIQNDLTSSNLQVEEDPVDGNSWKVSSQCADTRKVPDSSPATCHNNIMKQTMVDSSCRIL
 TSDVFDQCNKLVDPPEPYLDVCIYDTCSESIGDCACFCDTIAAYAHVCAQHKGKVVTVRTATLC
 PQSCEERNLRENGYCEWRYNSCAPACQVTCQHPEPLACPVCQVEGCHAHCPPGKILDELL
 QTCVDPEDCPVCEVAGRRFASGKKVTLNPSDPEHCQICHCDVNLTCACQEPGGGLVVPPTD
 APVSPTTLYVEDISEPPLHDFYCSRLDLVFLLDGSSRLSEAEFEVLKAFVDDMMERLRISQKW
 VRVAVVEYHDGSHAYIGLKDRKRPSELRRIASQVKYAGSQVASTSEVLKYTLFQIFSKIDRPEAS
 RIALLLMASQEPQRMSRNFVRYVQGLKKKKVIVIPVGIGPHANLKQIRLIEKQAPENKAFVLSSV
 DELEQQRDEIVSYLCDLAPEAPPPTLPPHMAQVTVGPGLLGVSTLGPKRNSMVLDAFVLEGS
 DKIGEADFNRSKEFMEEVIQRMVDVGQDSIHVTVLQYSYMTVEYFPFSEAQSKGDIQVRREIRY
 QGGNRTNTGLALRYLSDHSFLVSQGDREQAPNLVYMTGNPASDEIKRLPGDIQVVPVIGVGN
 ANVQELERIGWPNAPILIQDFETLPREAPDLVLQRCCSGEGLQIPTLSPAPDCSQPLDVILLDG
 SSSFPASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVPWNVVEKAHLLSLVDVMQR
 EGGPSQIGDALGFVRYLTSEMHGARPASKAVVILVTDVSVDSVDAADAARSNRVTVFPIGI
 GDRYDAAQLRILAGPAGDSNVVQLQRIEDLPTMTLGNLFLHKLCSGFVRICMDEDGNEKRP
 DVWTLPDQCHTVTCQPDGQTLKSHRVNCDRLRPSCPNSSQSPVKVEETCGCRWTCPCVCT
 GSSTRHIVTFDQGNFKLTGSCSYVLFQNKQEDLEVILHNGACSPGARQGCMMKSIEVKHSALSV
 ELHSDMEVTVNGRLVSPYVGGNMEVNVYGAIMHEVRFNHLGHIFTFTPQNNFEQLQLSPKTF
 ASKTYGLCGICDENGANDFMLRDGTVTDDWKTLLVQEWTVQRPQGTQCPILEEQCLVPDSSH
 QVLLLPLFAECHKVLAPATFYAICQQDSCHQEQQVEVIASHTALCRTNGVCVDWRTPDFCAMS
 CPPSLVYNHCEHGCPRHCDGNVSSCGDHPSEGCFCPPDKVMEGSCVPPEEACTQCIGEDGV
 QHQFLEAWVPDHQPCQICTCLSGRKVNCTTQPCPTAKAPTCLCEVARLRQNADQCCPEYEC
 VCDPVSCDLPPVPHCERGLQPTLTNPGECPNFTCACRKEECKRVSPSPCPPHRLPTLRKTQ
 CCDEYECACNCVNSTVSCPLGYLASTATNDGCGTTTTCLPDKVCVHRSTIYPVGQFWEEGCD
 VCTCTDMEDAVMGLRVAQCSQKPCEDSCRSQFTYVLHEGECCGRCLPSACEVVTGSPRGDS
 QSSWKSQVGSQWASPENPCLINECVRVKEEVFIQQRNVSCPQLEVPCPSGFLSCKTSACCP
 SCRCERMEACMLNGTVIGPGKTVMIDVCTTCRCMVQGVVISGFKLECRKTTCPNCPGLGYKEEN
 NTGECCGRCLPTACTIQLRGGQIMTLKRDETLQDGCDFHCKVNERGEYFWEKRVTGCPPFD
 EHKCLAEGGKIMKIPGTCCDTCEEPECNDITARLQYVKVGSCKSEVEVDIHYCQGKCAASKAMY
 SIDINDVQDQCCSCSPTRTEPMQVALHCTNGSVVYHEVLNAMECKCSPRKCSK
 (SEQ. ID. No. 7)

Fig. 4

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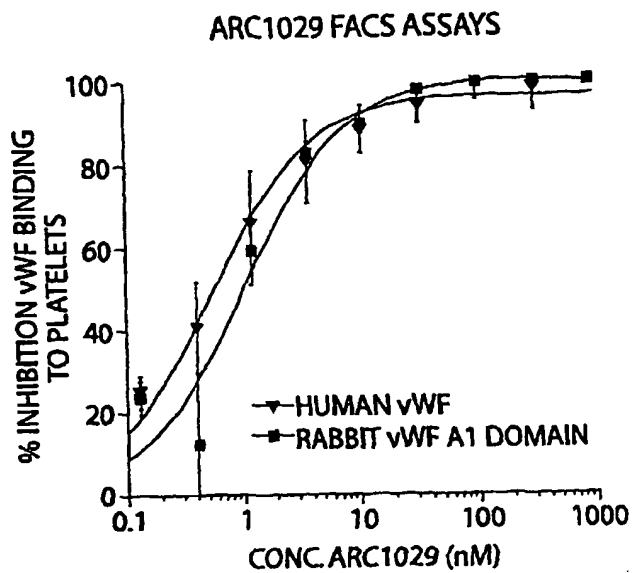


Fig. 7

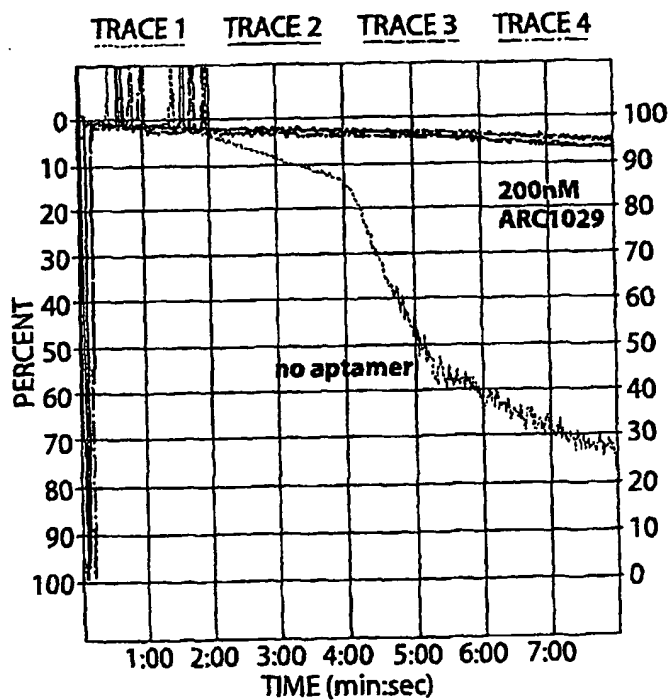


Fig. 8

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ARC1029 BIPA
3 REPS FROM EACH OF 2 DONORS

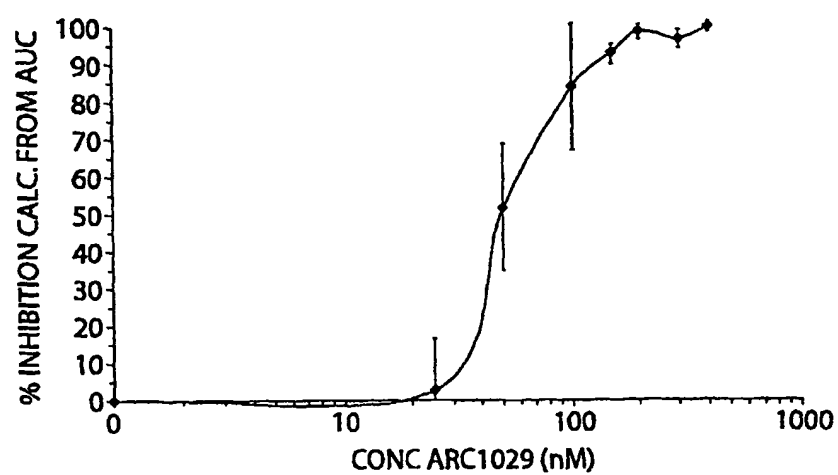


Fig. 9

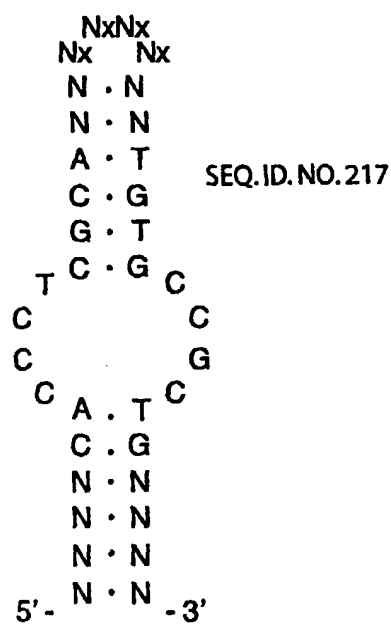


Fig. 10

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	Clone	70 min	3	90
	DL-159.87.70	(AMX203.G9)	32		32
	DL-159.87.75	(AMX203.F9)	61	CGACTCTCTGCTAGC	76
	DL-159.87.28	(AMX203.A6)	61	CGACTCTCTGCTAGC	76
	DL-159.87.28	(AMX203.A6)	61	CGACTCTCTGCTAGC	77

Fig. 11

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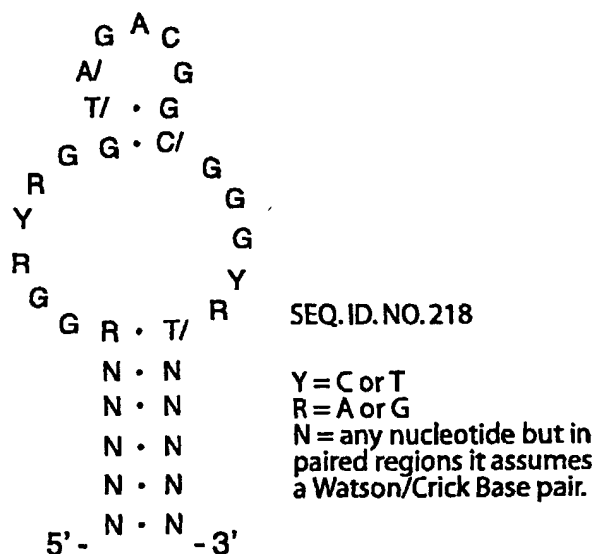


Fig. 12

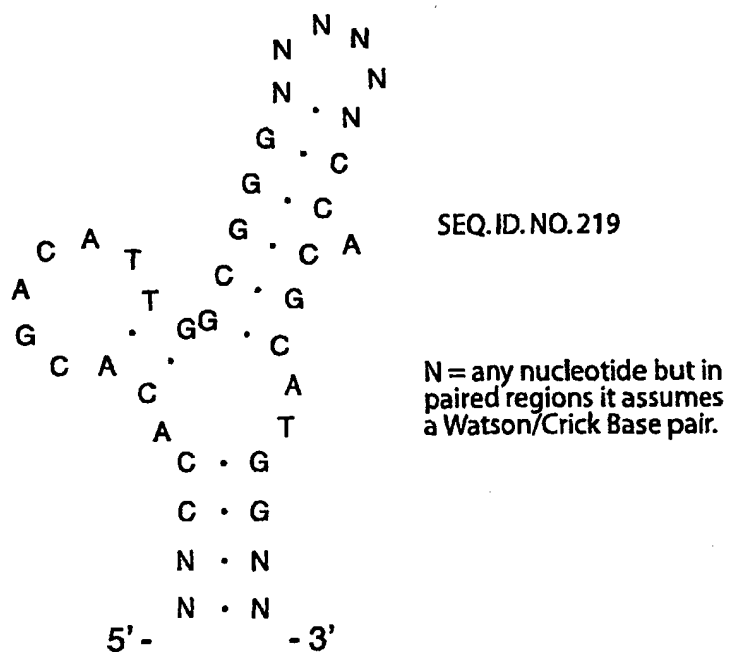


Fig. 13

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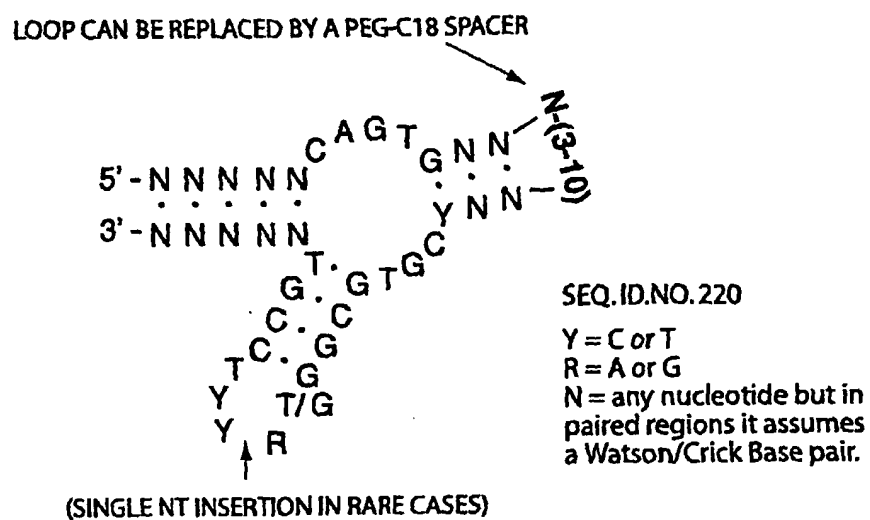


Fig. 15A

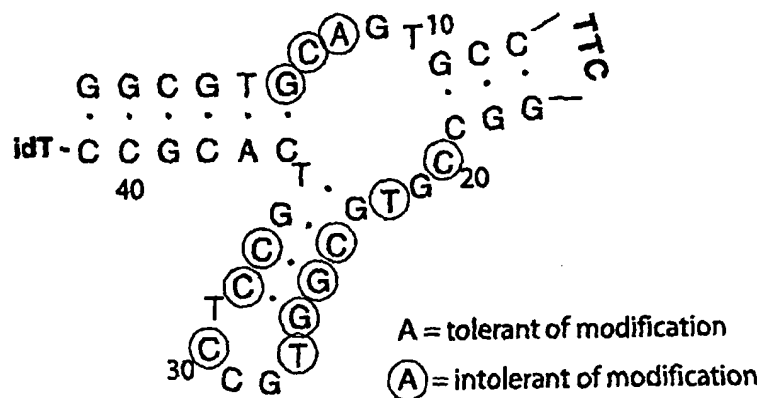


Fig. 15B

residue #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41		
	stem 1										stem 2						stem 2						stem 3									stem 3						stem 1					
ARC#	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	<	<	<	<	<	<	>	>	>	>	>	>	>	>	>	<	<	<	<	<	<	<	<	<	<	<	<	<
ARC1029	S	HG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	dG	dG	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3	
ARC1115	S	HG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1172	S	HG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1194	S	mG	dC	dG	dC	T	dG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1195	S	HG	mG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1196	S	HG	mG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1197	S	HG	dG	dC	mG	T	dG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1198	S	HG	dG	dC	dG	dG	mU	dG	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1199	S	HG	dG	dC	dC	T	mG	dC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1200	S	HG	dG	dC	dG	T	dG	mC	dA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1201	S	HG	dG	dC	dG	T	dG	dC	mA	dG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1202	S	HG	dG	dC	dG	T	dG	dC	dA	mG	T	dG	dC	dC	T	dC	dG	dC	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	(31)	3
ARC1203	S	HG	dG	dC	dG	dC	dG	T	dG	dC	mU	dG	dC	dC	T	dC	dG	dC	dC	d																							

Fig. 16

13/27

residue #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41		
	stem 1									stem 2						stem 2							stem 3								stem 3				stem 1								
ARC#	>	>	>	>	>	>				>	>	>				<	<	<					>	>	>						<	<	<	<	<	<	<	<	<	<	<		
ARC1172:5'	dG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	T	dC	dG	dG	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	[BT] 3'	
ARC1338:5'	mG	mC	mC	mG	mG	mG	dC	dA	dG	T	dG	dC	dC	T	T	dC	dG	dG	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1339:5'	dG	dG	dC	dG	T	dG	dC	dA	mG	mU	dG	dC	dC	T	T	dC	dG	dG	dC	dC	dG	T	mG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	mG	mU	dC	dA	dC	dG	dC	dC	[BT] 3'	
ARC1340:5'	dG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	T	dC	dG	dG	dC	dC	dG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	dA	dC	dG	dC	dC	[BT] 3'	
ARC1341:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1342:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1343:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1344:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1345:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	sI	mG	dC	dG	dG	sI	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1346:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1347:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1348:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1361:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1362:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1363:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1364:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1365:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1366:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1367:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1368:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	sI	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1369:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1370:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1371:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1372:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1373:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1374:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1375:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1376:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1377:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1378:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1379:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1380:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	
ARC1381:5'	mG	mC	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mU	mU	mC	mG	mG	mC	dC	mG	T	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	dC	mA	mC	mG	mC	mC	[BT] 3'	

Fig. 17

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residue #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41					
	stem 1									stem 2						stem 2							stem 3								stem 3					stem 1										
ARC#	>	>	>	>	>					>	>	>				<	<	<					>	>	>						<	<	<	<	<	<	<	<	<	<	<	<	<			
ARC1172	5'	dG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	T	dC	dG	dG	dC	dC	dG	T	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	[3T]	3'		
ARC1524	5'	mG	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'	
ARC1525	5'	mG	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'	
ARC1526	5'	mG	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'	
ARC1527	5'	mG	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'	
ARC1528	5'	mG	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'	
ARC1529	5'	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'		
ARC1530	5'	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'		
ARC1531	5'	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'		
ARC1532	5'	mC	mG	mU	dG	dC	dA	mG	mU	mG	mC	mU	mU	mC	mG	mG	mC	dC	mG	mU	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mC					[3T]	3'		
	stem 2									stem 3						stem 3						stem 1						stem 1						stem 2												
	>	>	>	>	>					>	>	>				<	<	<				>	>	>						<	<	<			<	<	<	<	<	<	<	<	<	<		
ARC1533	5'	mC	mG	mG	mC	dC	mG	sT	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mU	mU	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mG					[3T]	3'			
ARC1534	5'	mC	mG	mG	mC	dC	mG	sT	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mU	mU	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mG					[3T]	3'			
ARC1535	5'	mC	mG	mG	mC	dC	mG	sT	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mU	mU	mG	mU	dG	dC	dA	mG	mU	mG	mC	mC	mG					[3T]	3'			
	stem 2									stem 3						stem 3						stem 1						stem 1						stem 2												
	>	>	>	>						>	>	>				<	<	<				>	>	>						<	<	<			<	<	<	<	<	<	<	<	<	<	<	
ARC1548	5'	mC	mG	mG	mC	dC	mG	sT	mG	dC	dG	dG	T	mG	mC	dC	mU	dC	dC	mG	mU	mC	mA	mC	mG	mU	mU	mG	mU	dC	dC	dA	mG	mU	mG	mC	mC	mG					[3T]	3'		
	stem 1									stem 2						stem 2						stem 3						stem 3						stem 1												
	>	>	>	>	>					>	>	>				<	<	<				>	>	>						<	<	<			<	<	<	<	<	<	<	<	<	<	<	<
ARC1549	5'	dG	dG	dC	dG	T	dG	dC	dA	dG	T	dG	dC	dC	T	T	dC	dG	dG	dC	dC	dG	sT	dG	dC	dG	dG	T	dG	dC	dC	T	dC	dC	dG	T	dC	dA	dC	dG	dC	dC	[3T]	3'		

Fig. 18

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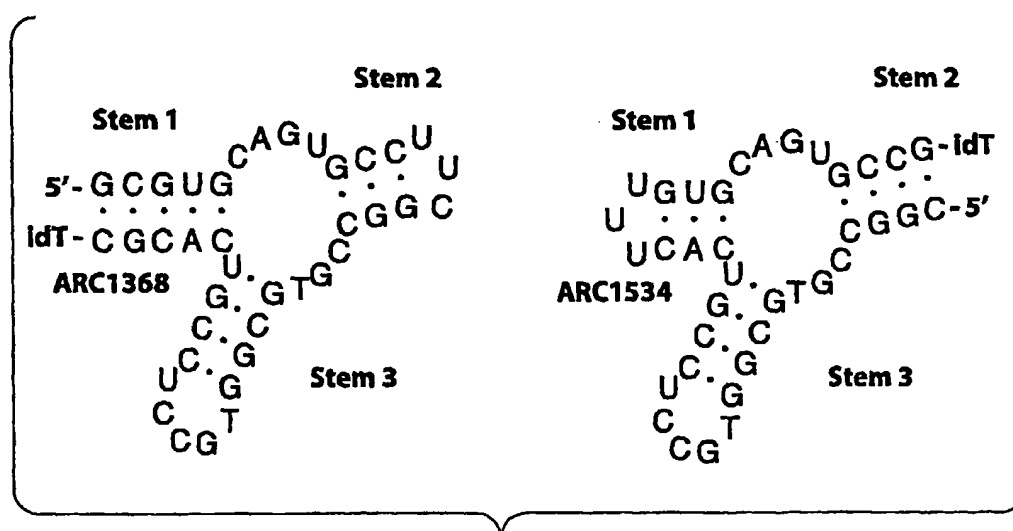


Fig. 19

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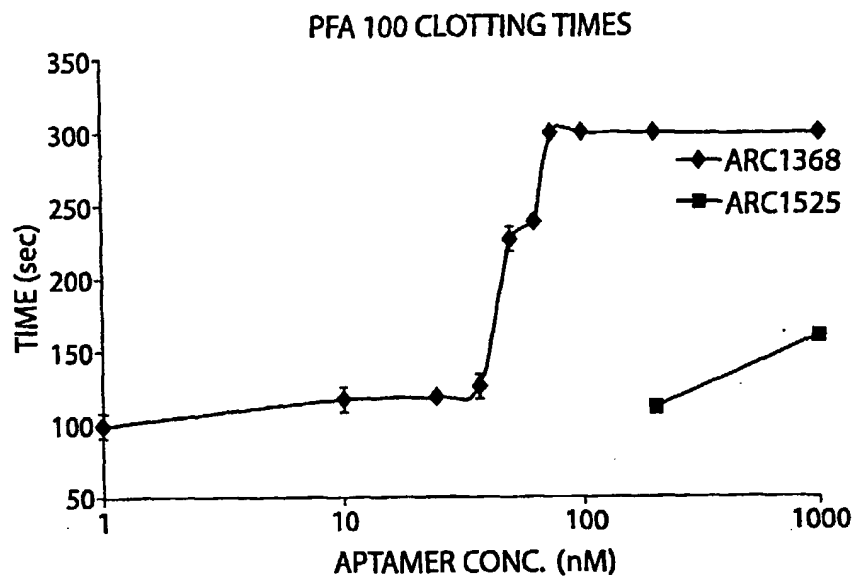


Fig. 20

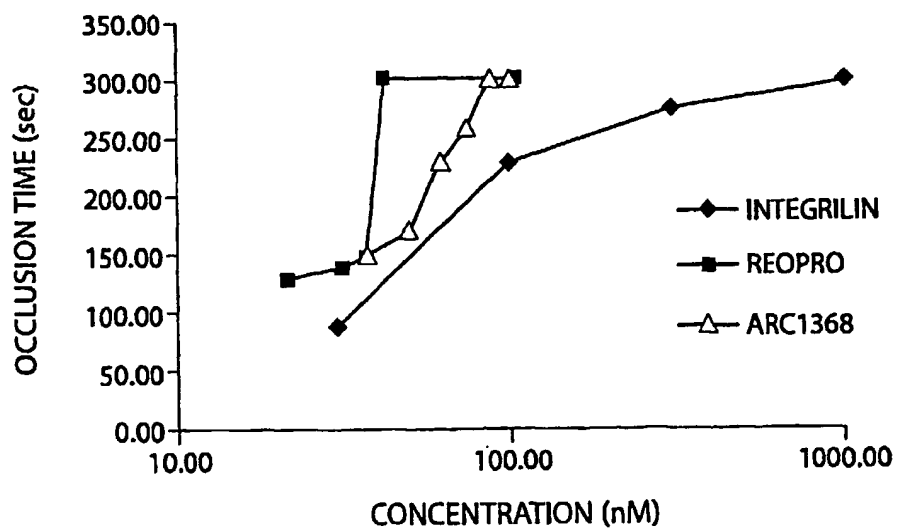


Fig. 21

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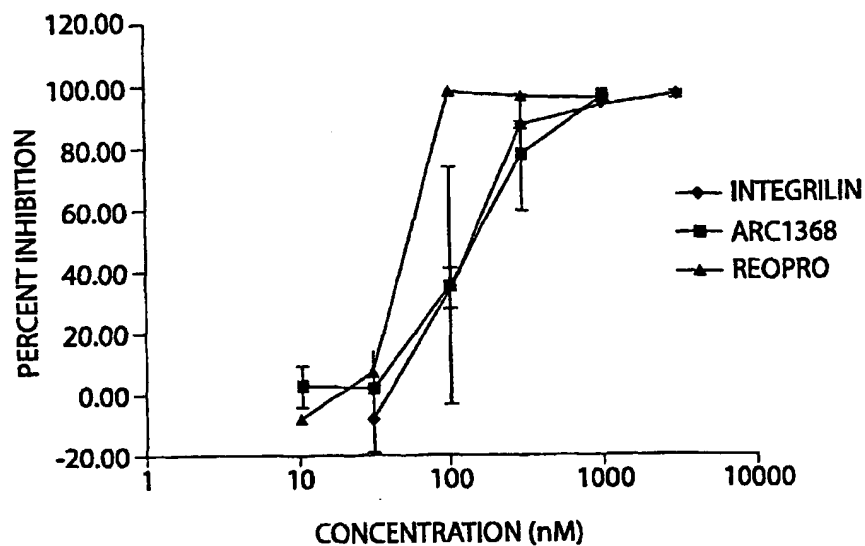


Fig. 22

TITRATION IN AIPA

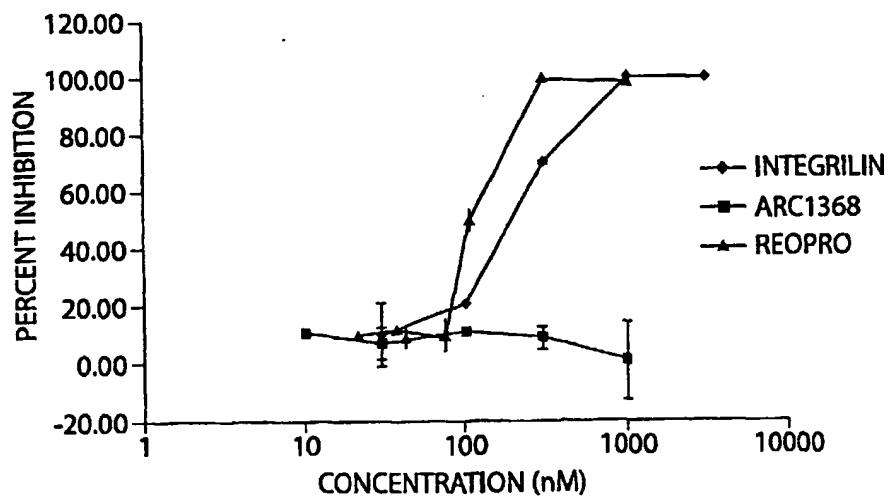


Fig. 23

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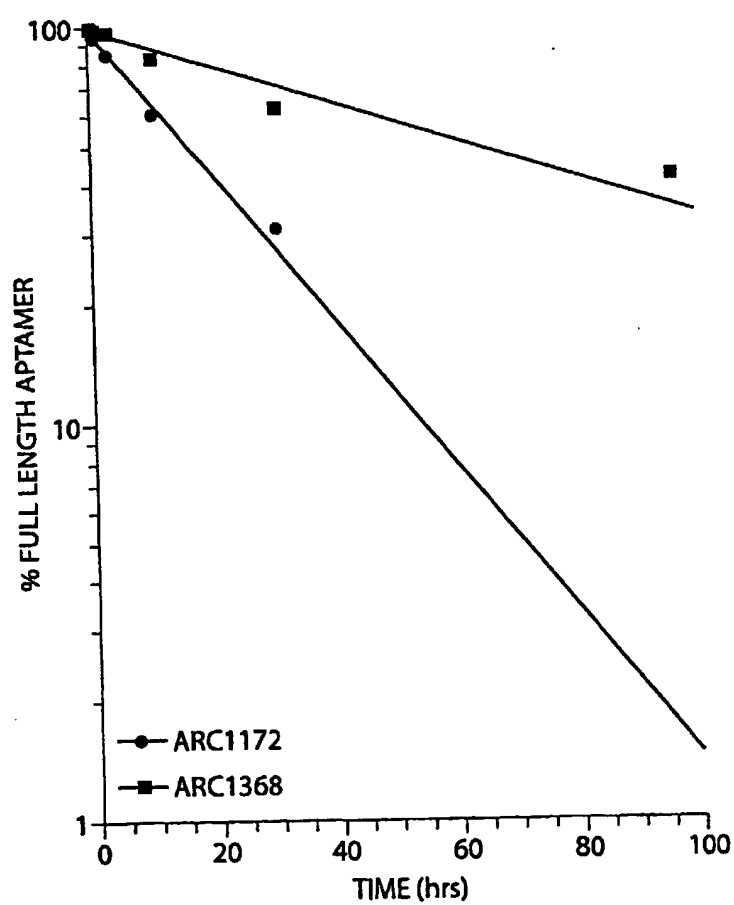


Fig. 24

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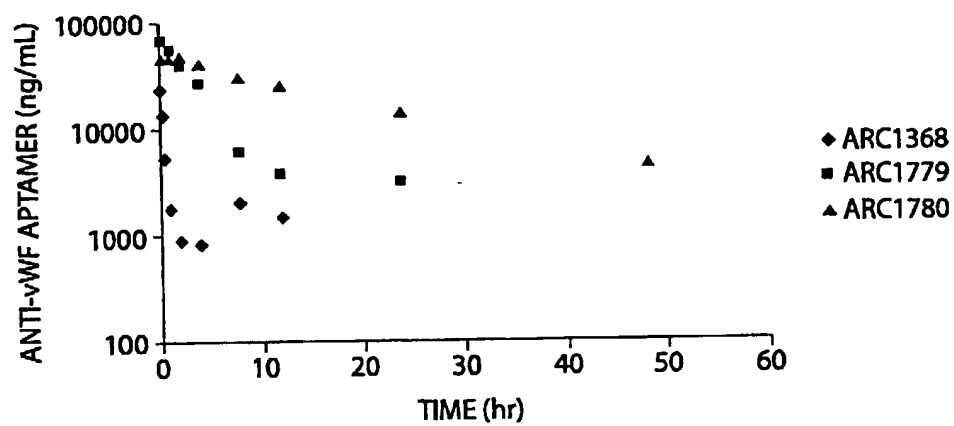


Fig. 25

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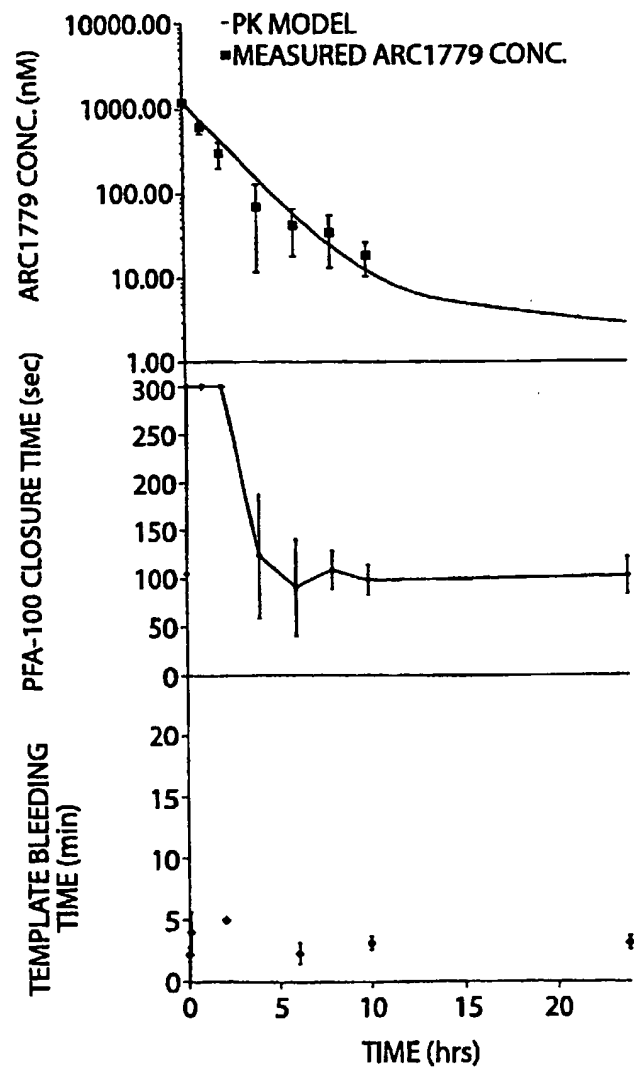


Fig. 26

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	ANIMAL 1001			ANIMAL 1101			ANIMAL 1102		
	CBT	BIPA	PFA-100	CBT	BIPA	PFA-100	CBT	BIPA	PFA-100
Pre	1	30	79	3	36	124	2	34	120
5 min	3.2	>>	>>	6.8	>>	>>	4.8	>>	>>
6 hours	1		119	1.3		107	2.3		163
10 hours	2.4		99	3		107	3.5		155
24 hours	2.3	47	92	2.3	51	106	1.8	NA	NA

>> = 100% INHIBITION

Fig. 27

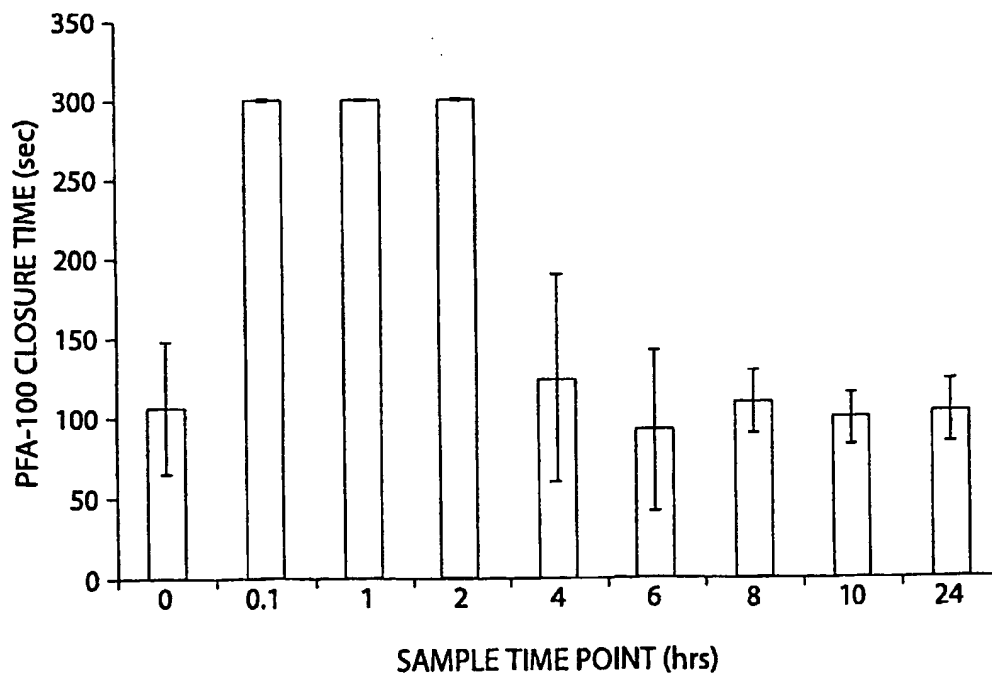


Fig. 28

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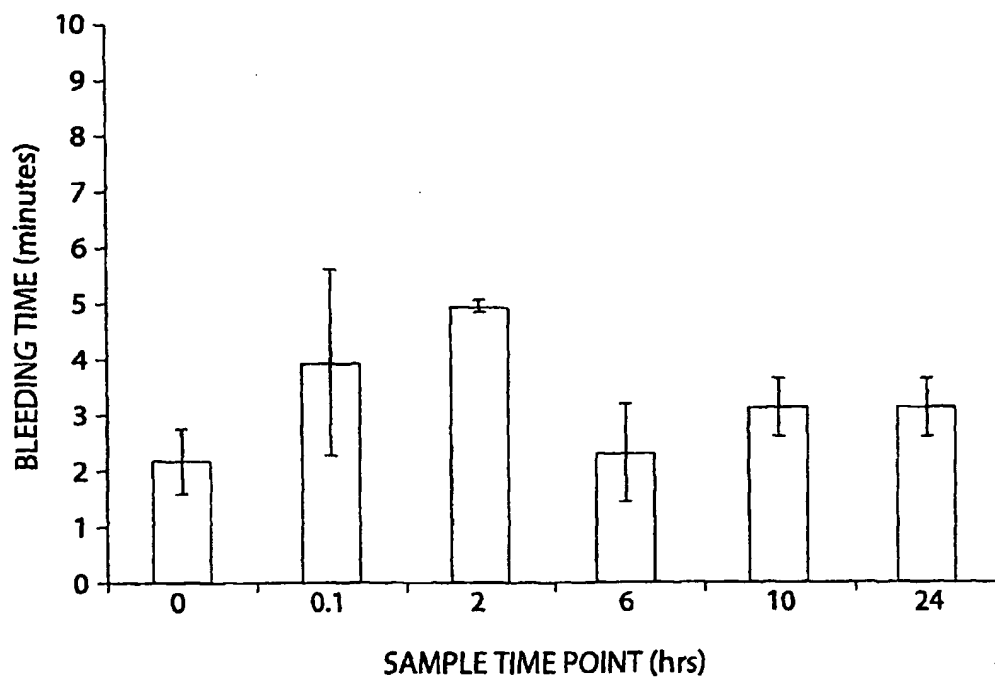


Fig. 29

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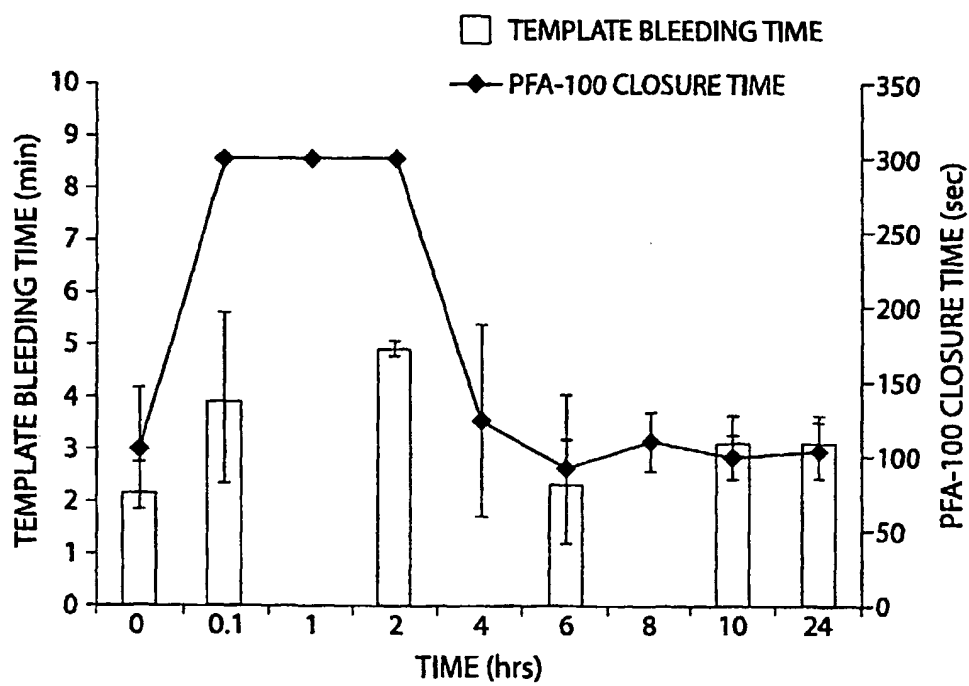


Fig. 30

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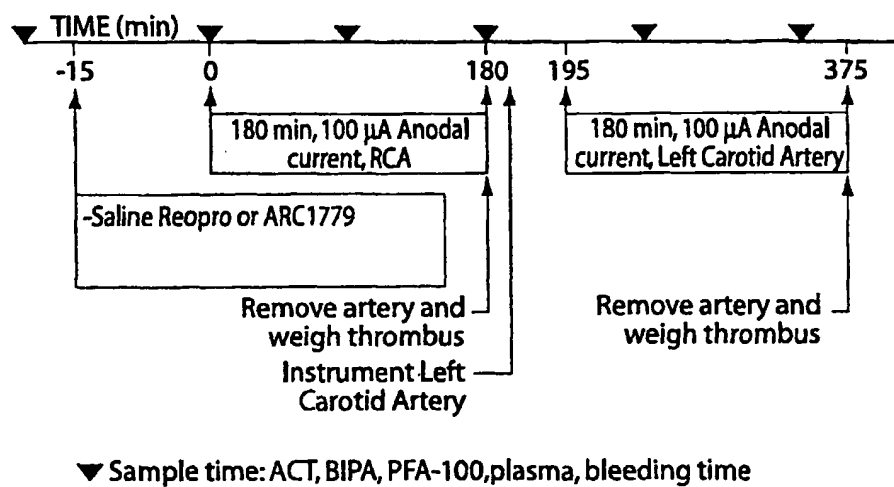


Fig. 31

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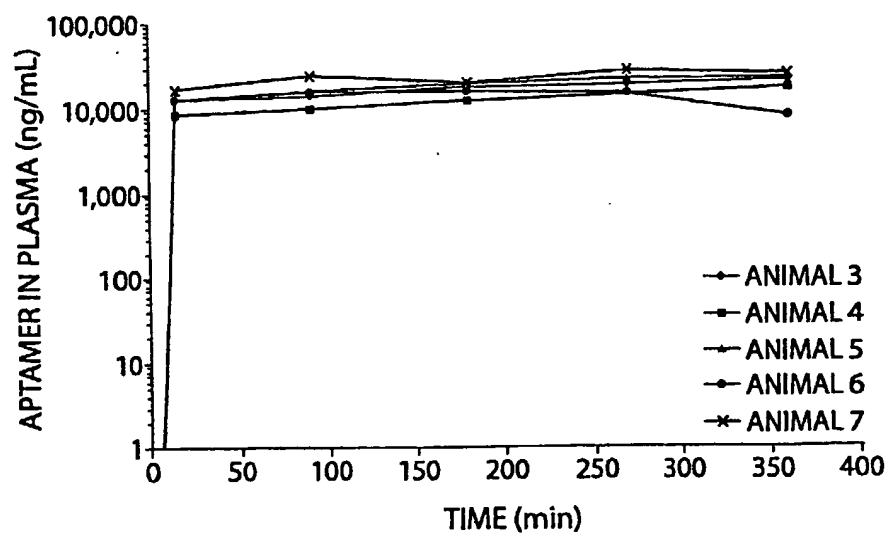


Fig. 32

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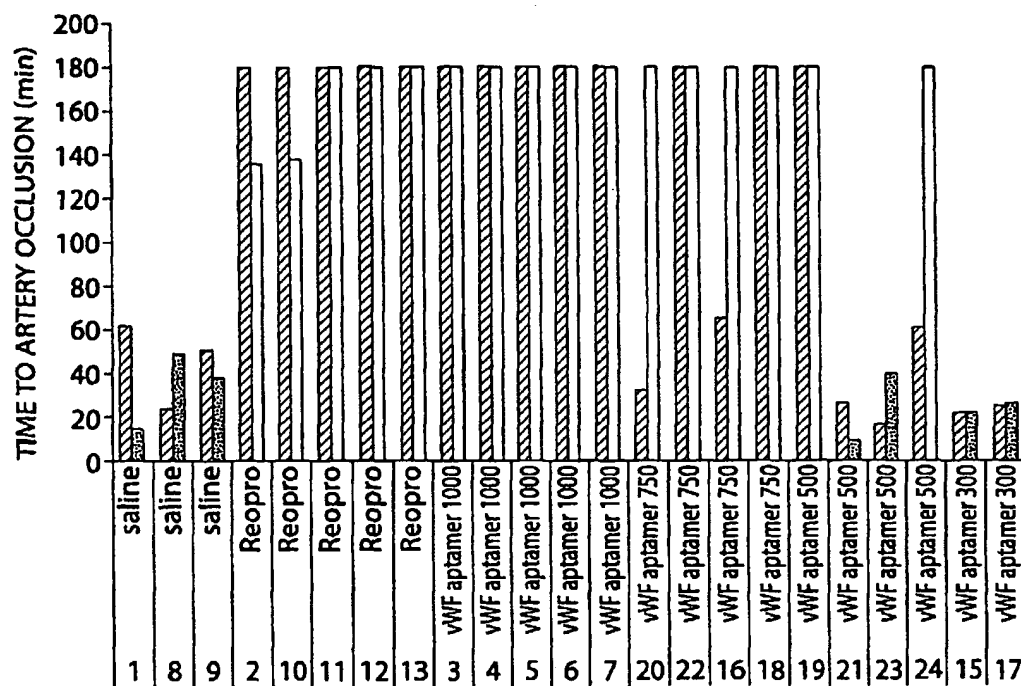


Fig. 33

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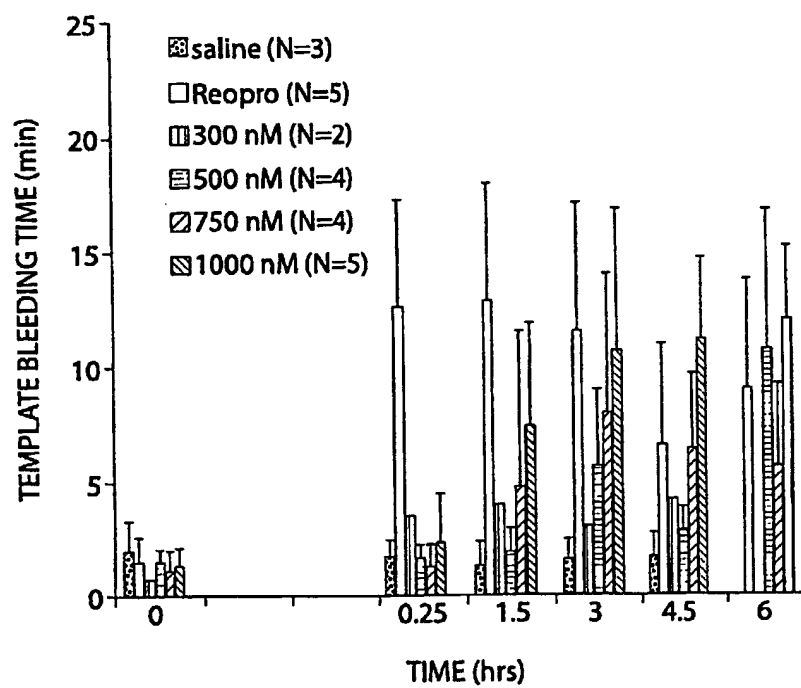


Fig. 34

SEQUENCE LISTING

<110> Archemix Corp., et al.

<120> Aptamers to von Willebrand Factor And their Use As Thrombotic Disease Therapeutics

<130> 23239-582-061

<150> 60/608,047

<151> 2004-09-07

<150> 60/661,950

<151> 2005-03-11

<150> 60/678,427

<151> 2005-05-06

<150> 60/690,231

<151> 2005-06-13

<160> 327

<170> PatentIn version 3.2

<210> 1

<211> 93

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<223> n may be any nucleotide (A, T, C or G)

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<223> n is a, c, g, or t

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cggtctctcc tctccctata gtgagtcgta tta 93

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<213> Artificial

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<223> Description of Artificial Sequence: chemically synthesized

<220>

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gttctctcct ctccctatag tgagtcgtat ta 92

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<212> DNA

<213> Artificial

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<223> Description of Artificial Sequence: chemically synthesized

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<221> misc_feature

<222> (24)..(53)

<223> n may be any nucleotide (A, T, C or G)

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catcgatcga tcgatcgaca gcgnnnnnnnn nnnnnnnnnnn nnnnnnnnnnn nnngtagaac 60

gttctctcct ctccctatag tgagtcgtat ta 92

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<211> 213

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<213> Artificial

<220>

<223> chemically synthesized

<400> 4

Met	Gly	His	His	His	His	His	His	Glu	Pro	Pro	Leu	His	Asp	Phe	Tyr
1									10					15	

Cys	Ser	Arg	Leu	Leu	Asp	Leu	Val	Phe	Leu	Leu	Asp	Gly	Ser	Ser	Arg
			20					25					30		

Leu	Ser	Glu	Ala	Glu	Phe	Glu	Val	Leu	Lys	Ala	Phe	Val	Val	Asp	Met
			35					40					45		

Met Glu Gln Leu Arg Ile Ser Gln Lys Trp Val Arg Val Ala Val Val
 50 55 60
 Glu Tyr His Asp Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys
 65 70 75 80
 Arg Pro Ser Glu Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly
 85 90 95
 Ser Gln Val Ala Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln
 100 105 110
 Ile Phe Ser Lys Ile Asp Arg Pro Glu Ala Ser Arg Ile Ala Leu Leu
 115 120 125
 Leu Met Ala Ser Gln Glu Pro Gln Arg Met Ser Arg Asn Phe Val Arg
 130 135 140
 Tyr Val Gln Gly Leu Lys Lys Lys Lys Val Ile Val Ile Pro Val Gly
 145 150 155 160
 Ile Gly Pro His Ala Asn Leu Lys Gln Ile Arg Leu Ile Glu Lys Gln
 165 170 175
 Ala Pro Glu Asn Lys Ala Phe Val Leu Ser Ser Val Asp Glu Leu Glu
 180 185 190
 Gln Gln Arg Asp Glu Ile Val Ser Tyr Leu Cys Asp Leu Ala Pro Glu
 195 200 205
 Ala Pro Pro Pro Thr
 210

<210> 5
 <211> 246
 <212> PRT
 <213> Artificial
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Gly	Leu	Val	Val	Pro	Pro	Thr	Asp	Ala	Pro	Val	Ser	Pro	Thr	Thr	Leu				
		20						25					30						
Tyr	Val	Glu	Asp	Ile	Ser	Glu	Pro	Pro	Leu	His	Asp	Phe	Tyr	Cys	Ser				
		35					40					45							
Arg	Leu	Leu	Asp	Leu	Val	Phe	Leu	Leu	Asp	Gly	Ser	Ser	Arg	Leu	Ser				
		50				55					60								
Glu	Ala	Glu	Phe	Glu	Val	Leu	Lys	Ala	Phe	Val	Val	Asp	Met	Met	Glu				
65					70					75					80				
Arg	Leu	Arg	Ile	Ser	Gln	Lys	Trp	Val	Arg	Val	Ala	Val	Val	Glu	Tyr				
				85					90					95					
His	Asp	Gly	Ser	His	Ala	Tyr	Ile	Gly	Leu	Lys	Asp	Arg	Lys	Arg	Pro				
			100					105					110						
Ser	Glu	Leu	Arg	Arg	Ile	Ala	Ser	Gln	Val	Lys	Tyr	Ala	Gly	Ser	Gln				
		115					120					125							
Val	Ala	Ser	Thr	Ser	Glu	Val	Leu	Lys	Tyr	Thr	Leu	Phe	Gln	Ile	Phe				
	130					135					140								
Ser	Lys	Ile	Asp	Arg	Pro	Glu	Ala	Ser	Arg	Ile	Ala	Leu	Leu	Leu	Met				
145					150					155					160				
Ala	Ser	Gln	Glu	Pro	Gln	Arg	Met	Ser	Arg	Asn	Phe	Val	Arg	Tyr	Val				
				165					170					175					
Gln	Gly	Leu	Lys	Lys	Lys	Lys	Val	Ile	Val	Ile	Pro	Val	Gly	Ile	Gly				
		180						185					190						
Pro	His	Ala	Asn	Leu	Lys	Gln	Ile	Arg	Leu	Ile	Glu	Lys	Gln	Ala	Pro				
		195					200					205							
Glu	Asn	Lys	Ala	Phe	Val	Leu	Ser	Ser	Val	Asp	Glu	Leu	Glu	Gln	Gln				
210						215					220								
Arg	Asp	Glu	Ile	Val	Ser	Tyr	Leu	Cys	Asp	Leu	Ala	Pro	Glu	Ala	Pro				

225 230 235 240

Pro Pro Thr Leu Pro Pro
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<211> 213
<212> PRT
<213> Artificial
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Trp Ser Asn Leu Met Asp Leu Val Phe Leu Leu Asp Gly Ser Ala Gln
20 25 30

Leu Ser Glu Ala Glu Phe Gly Val Leu Lys Ala Phe Val Val Ser Val
35 40 45

Met Glu Arg Leu His Ile Ser Gln Lys Arg Ile Arg Val Ala Val Val
50 55 60

Glu Tyr His Asp Gly Ser His Ser Tyr Ile Ser Leu Lys Asp Arg Lys
65 70 75 80

Arg Pro Ser Glu Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly
85 90 95

Gly Pro Val Ala Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe His
100 105 110

Ile Phe Ser Asn Val Asp Arg Pro Glu Ala Ser Arg Ile Ala Leu Leu
115 120 125

Leu Ser Ala Ser Gln Glu Thr Pro Arg Met Val Arg Asn Leu Val Arg
130 135 140

Tyr Ala Gln Gly Leu Lys Lys Glu Lys Val Ile Val Ile Pro Val Gly
145 150 155 160

Ile Gly Pro His Val Ser Leu Arg Gln Ile His Leu Ile Glu Lys Gln
 165 170 175

Ala Pro Glu Asn Lys Ala Phe Val Leu Ser Gly Val Asp Glu Leu Glu
 180 185 190

Gln Arg Arg Asp Glu Ile Ile Ser Tyr Leu Cys Asp Leu Gly Pro Glu
 195 200 205

Ala Pro Val Pro Thr
 210

<210> 7
 <211> 2813
 <212> PRT
 <213> Artificial

<220>
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Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr
 20 25 30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly
 35 40 45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly
 50 55 60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys
 65 70 75 80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu
 85 90 95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro
 100 105 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys

115	120	125
Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly		
130	135	140
Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly		
145	150	155
		160
Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln		
	165	170
		175
Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala		
180	185	190
Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser		
195	200	205
Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln		
210	215	220
Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu		
225	230	235
		240
Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu		
	245	250
		255
Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala		
260	265	270
Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His		
275	280	285
Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys		
290	295	300
Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met		
305	310	315
		320
Cys Gln Glu Arg Cys Val Asp Gly Cys Ser Cys Pro Glu Gly Gln Leu		
325	330	335
Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys Pro Cys Val His		

340	345	350
Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser Arg Asp Cys Asn		
355	360	365
Thr Cys Ile Cys Arg Asn Ser Gln Trp Ile Cys Ser Asn Glu Glu Cys		
370	375	380
Pro Gly Glu Cys Leu Val Thr Gly Gln Ser His Phe Lys Ser Phe Asp		
385	390	395
Asn Arg Tyr Phe Thr Phe Ser Gly Ile Cys Gln Tyr Leu Leu Ala Arg		
405	410	415
Asp Cys Gln Asp His Ser Phe Ser Ile Val Ile Glu Thr Val Gln Cys		
420	425	430
Ala Asp Asp Arg Asp Ala Val Cys Thr Arg Ser Val Thr Val Arg Leu		
435	440	445
Pro Gly Leu His Asn Ser Leu Val Lys Leu Lys His Gly Ala Gly Val		
450	455	460
Ala Met Asp Gly Gln Asp Ile Gln Leu Pro Leu Leu Lys Gly Asp Leu		
465	470	475
Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu		
485	490	495
Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu		
500	505	510
Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn		
515	520	525
Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro		
530	535	540
Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln		
545	550	555
Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met		

565	570	575
Thr Arg Phe Ser Glu Glu Ala Cys	Ala Val Leu Thr Ser Pro Thr Phe	
580	585	590
Glu Ala Cys His Arg Ala Val Ser	Pro Leu Pro Tyr Leu Arg Asn Cys	
595	600	605
Arg Tyr Asp Val Cys Ser Cys Ser	Asp Gly Arg Glu Cys Leu Cys Gly	
610	615	620
Ala Leu Ala Ser Tyr Ala Ala Ala Cys	Ala Gly Arg Gly Val Arg Val	
625	630	635
Ala Trp Arg Glu Pro Gly Arg Cys Glu	Leu Asn Cys Pro Lys Gly Gln	
645	650	655
Val Tyr Leu Gln Cys Gly Thr Pro Cys	Asn Leu Thr Cys Arg Ser Leu	
660	665	670
Ser Tyr Pro Asp Glu Glu Cys Asn Glu	Ala Cys Leu Glu Gly Cys Phe	
675	680	685
Cys Pro Pro Gly Leu Tyr Met Asp Glu	Arg Gly Asp Cys Val Pro Lys	
690	695	700
Ala Gln Cys Pro Cys Tyr Tyr Asp Gly	Glu Ile Phe Gln Pro Glu Asp	
705	710	715
Ile Phe Ser Asp His His Thr Met Cys	Tyr Cys Glu Asp Gly Phe Met	
725	730	735
His Cys Thr Met Ser Gly Val Pro Gly	Ser Leu Leu Pro Asp Ala Val	
740	745	750
Leu Ser Ser Pro Leu Ser His Arg Ser	Lys Arg Ser Leu Ser Cys Arg	
755	760	765
Pro Pro Met Val Lys Leu Val Cys Pro	Ala Asp Asn Leu Arg Ala Glu	
770	775	780
Gly Leu Glu Cys Thr Lys Thr Cys Gln	Asn Tyr Asp Leu Glu Cys Met	

10

1010		1015		1020
Ser Trp	Lys Val Ser Ser	Gln Cys Ala Asp Thr	Arg Lys Val Pro	
1025		1030	1035	
Leu Asp	Ser Ser Pro Ala Thr	Cys His Asn Asn Ile	Met Lys Gln	
1040		1045	1050	
Thr Met	Val Asp Ser Ser Cys	Arg Ile Leu Thr Ser	Asp Val Phe	
1055		1060	1065	
Gln Asp	Cys Asn Lys Leu Val	Asp Pro Glu Pro Tyr	Leu Asp Val	
1070		1075	1080	
Cys Ile	Tyr Asp Thr Cys Ser	Cys Glu Ser Ile Gly	Asp Cys Ala	
1085		1090	1095	
Cys Phe	Cys Asp Thr Ile Ala	Ala Tyr Ala His Val	Cys Ala Gln	
1100		1105	1110	
His Gly	Lys Val Val Thr Trp	Arg Thr Ala Thr Leu	Cys Pro Gln	
1115		1120	1125	
Ser Cys	Glu Glu Arg Asn Leu	Arg Glu Asn Gly Tyr	Glu Cys Glu	
1130		1135	1140	
Trp Arg	Tyr Asn Ser Cys Ala	Pro Ala Cys Gln Val	Thr Cys Gln	
1145		1150	1155	
His Pro	Glu Pro Leu Ala Cys	Pro Val Gln Cys Val	Glu Gly Cys	
1160		1165	1170	
His Ala	His Cys Pro Pro Gly	Lys Ile Leu Asp Glu	Leu Leu Gln	
1175		1180	1185	
Thr Cys	Val Asp Pro Glu Asp	Cys Pro Val Cys Glu	Val Ala Gly	
1190		1195	1200	
Arg Arg	Phe Ala Ser Gly Lys	Lys Val Thr Leu Asn	Pro Ser Asp	
1205		1210	1215	
Pro Glu	His Cys Gln Ile Cys	His Cys Asp Val Val	Asn Leu Thr	

1220		1225		1230
Cys Glu Ala Cys Gln Glu Pro Gly Gly Leu Val Val Pro Pro Thr				
1235		1240		1245
Asp Ala Pro Val Ser Pro Thr Thr Leu Tyr Val Glu Asp Ile Ser				
1250		1255		1260
Glu Pro Pro Leu His Asp Phe Tyr Cys Ser Arg Leu Leu Asp Leu				
1265		1270		1275
Val Phe Leu Leu Asp Gly Ser Ser Arg Leu Ser Glu Ala Glu Phe				
1280		1285		1290
Glu Val Leu Lys Ala Phe Val Val Asp Met Met Glu Arg Leu Arg				
1295		1300		1305
Ile Ser Gln Lys Trp Val Arg Val Ala Val Val Glu Tyr His Asp				
1310		1315		1320
Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys Arg Pro Ser				
1325		1330		1335
Glu Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly Ser Gln				
1340		1345		1350
Val Ala Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln Ile				
1355		1360		1365
Phe Ser Lys Ile Asp Arg Pro Glu Ala Ser Arg Ile Ala Leu Leu				
1370		1375		1380
Leu Met Ala Ser Gln Glu Pro Gln Arg Met Ser Arg Asn Phe Val				
1385		1390		1395
Arg Tyr Val Gln Gly Leu Lys Lys Lys Lys Val Ile Val Ile Pro				
1400		1405		1410
Val Gly Ile Gly Pro His Ala Asn Leu Lys Gln Ile Arg Leu Ile				
1415		1420		1425
Glu Lys Gln Ala Pro Glu Asn Lys Ala Phe Val Leu Ser Ser Val				

1430	1435	1440
Asp Glu Leu Glu Gln Gln Arg	Asp Glu Ile Val Ser	Tyr Leu Cys
1445	1450	1455
Asp Leu Ala Pro Glu Ala Pro	Pro Pro Thr Leu Pro	Pro His Met
1460	1465	1470
Ala Gln Val Thr Val Gly Pro	Gly Leu Leu Gly Val	Ser Thr Leu
1475	1480	1485
Gly Pro Lys Arg Asn Ser Met	Val Leu Asp Val Ala	Phe Val Leu
1490	1495	1500
Glu Gly Ser Asp Lys Ile Gly	Glu Ala Asp Phe Asn	Arg Ser Lys
1505	1510	1515
Glu Phe Met Glu Glu Val Ile	Gln Arg Met Asp Val	Gly Gln Asp
1520	1525	1530
Ser Ile His Val Thr Val Leu	Gln Tyr Ser Tyr Met	Val Thr Val
1535	1540	1545
Glu Tyr Pro Phe Ser Glu Ala	Gln Ser Lys Gly Asp	Ile Leu Gln
1550	1555	1560
Arg Val Arg Glu Ile Arg Tyr	Gln Gly Gly Asn Arg	Thr Asn Thr
1565	1570	1575
Gly Leu Ala Leu Arg Tyr Leu	Ser Asp His Ser Phe	Leu Val Ser
1580	1585	1590
Gln Gly Asp Arg Glu Gln Ala	Pro Asn Leu Val Tyr	Met Val Thr
1595	1600	1605
Gly Asn Pro Ala Ser Asp Glu	Ile Lys Arg Leu Pro	Gly Asp Ile
1610	1615	1620
Gln Val Val Pro Ile Gly Val	Gly Pro Asn Ala Asn	Val Gln Glu
1625	1630	1635
Leu Glu Arg Ile Gly Trp Pro	Asn Ala Pro Ile Leu	Ile Gln Asp

1640	1645	1650
Phe Glu Thr Leu Pro Arg Glu Ala Pro Asp Leu Val Leu Gln Arg		
1655	1660	1665
Cys Cys Ser Gly Glu Gly Leu Gln Ile Pro Thr Leu Ser Pro Ala		
1670	1675	1680
Pro Asp Cys Ser Gln Pro Leu Asp Val Ile Leu Leu Leu Asp Gly		
1685	1690	1695
Ser Ser Ser Phe Pro Ala Ser Tyr Phe Asp Glu Met Lys Ser Phe		
1700	1705	1710
Ala Lys Ala Phe Ile Ser Lys Ala Asn Ile Gly Pro Arg Leu Thr		
1715	1720	1725
Gln Val Ser Val Leu Gln Tyr Gly Ser Ile Thr Thr Ile Asp Val		
1730	1735	1740
Pro Trp Asn Val Val Pro Glu Lys Ala His Leu Leu Ser Leu Val		
1745	1750	1755
Asp Val Met Gln Arg Glu Gly Gly Pro Ser Gln Ile Gly Asp Ala		
1760	1765	1770
Leu Gly Phe Ala Val Arg Tyr Leu Thr Ser Glu Met His Gly Ala		
1775	1780	1785
Arg Pro Gly Ala Ser Lys Ala Val Val Ile Leu Val Thr Asp Val		
1790	1795	1800
Ser Val Asp Ser Val Asp Ala Ala Ala Asp Ala Ala Arg Ser Asn		
1805	1810	1815
Arg Val Thr Val Phe Pro Ile Gly Ile Gly Asp Arg Tyr Asp Ala		
1820	1825	1830
Ala Gln Leu Arg Ile Leu Ala Gly Pro Ala Gly Asp Ser Asn Val		
1835	1840	1845
Val Lys Leu Gln Arg Ile Glu Asp Leu Pro Thr Met Val Thr Leu		

1850	1855	1860
Gly Asn Ser Phe Leu His Lys	Leu Cys Ser Gly Phe	Val Arg Ile
1865	1870	1875
Cys Met Asp Glu Asp Gly Asn	Glu Lys Arg Pro Gly	Asp Val Trp
1880	1885	1890
Thr Leu Pro Asp Gln Cys His	Thr Val Thr Cys Gln	Pro Asp Gly
1895	1900	1905
Gln Thr Leu Leu Lys Ser His	Arg Val Asn Cys Asp	Arg Gly Leu
1910	1915	1920
Arg Pro Ser Cys Pro Asn Ser	Gln Ser Pro Val Lys	Val Glu Glu
1925	1930	1935
Thr Cys Gly Cys Arg Trp Thr	Cys Pro Cys Val Cys	Thr Gly Ser
1940	1945	1950
Ser Thr Arg His Ile Val Thr	Phe Asp Gly Gln Asn	Phe Lys Leu
1955	1960	1965
Thr Gly Ser Cys Ser Tyr Val	Leu Phe Gln Asn Lys	Glu Gln Asp
1970	1975	1980
Leu Glu Val Ile Leu His Asn	Gly Ala Cys Ser Pro	Gly Ala Arg
1985	1990	1995
Gln Gly Cys Met Lys Ser Ile	Glu Val Lys His Ser	Ala Leu Ser
2000	2005	2010
Val Glu Leu His Ser Asp Met	Glu Val Thr Val Asn	Gly Arg Leu
2015	2020	2025
Val Ser Val Pro Tyr Val Gly	Gly Asn Met Glu Val	Asn Val Tyr
2030	2035	2040
Gly Ala Ile Met His Glu Val	Arg Phe Asn His Leu	Gly His Ile
2045	2050	2055
Phe Thr Phe Thr Pro Gln Asn	Asn Glu Phe Gln Leu	Gln Leu Ser

2060		2065		2070
Pro Lys Thr Phe Ala Ser Lys Thr Tyr Gly Leu Cys Gly Ile Cys				
2075		2080		2085
Asp Glu Asn Gly Ala Asn Asp Phe Met Leu Arg Asp Gly Thr Val				
2090		2095		2100
Thr Thr Asp Trp Lys Thr Leu Val Gln Glu Trp Thr Val Gln Arg				
2105		2110		2115
Pro Gly Gln Thr Cys Gln Pro Ile Leu Glu Glu Gln Cys Leu Val				
2120		2125		2130
Pro Asp Ser Ser His Cys Gln Val Leu Leu Leu Pro Leu Phe Ala				
2135		2140		2145
Glu Cys His Lys Val Leu Ala Pro Ala Thr Phe Tyr Ala Ile Cys				
2150		2155		2160
Gln Gln Asp Ser Cys His Gln Glu Gln Val Cys Glu Val Ile Ala				
2165		2170		2175
Ser Tyr Ala His Leu Cys Arg Thr Asn Gly Val Cys Val Asp Trp				
2180		2185		2190
Arg Thr Pro Asp Phe Cys Ala Met Ser Cys Pro Pro Ser Leu Val				
2195		2200		2205
Tyr Asn His Cys Glu His Gly Cys Pro Arg His Cys Asp Gly Asn				
2210		2215		2220
Val Ser Ser Cys Gly Asp His Pro Ser Glu Gly Cys Phe Cys Pro				
2225		2230		2235
Pro Asp Lys Val Met Leu Glu Gly Ser Cys Val Pro Glu Glu Ala				
2240		2245		2250
Cys Thr Gln Cys Ile Gly Glu Asp Gly Val Gln His Gln Phe Leu				
2255		2260		2265
Glu Ala Trp Val Pro Asp His Gln Pro Cys Gln Ile Cys Thr Cys				

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Leu Ser Gly Arg Lys Val Asn Cys Thr Thr Gln Pro Cys Pro Thr		
2285	2290	2295
Ala Lys Ala Pro Thr Cys Gly Leu Cys Glu Val Ala Arg Leu Arg		
2300	2305	2310
Gln Asn Ala Asp Gln Cys Cys Pro Glu Tyr Glu Cys Val Cys Asp		
2315	2320	2325
Pro Val Ser Cys Asp Leu Pro Pro Val Pro His Cys Glu Arg Gly		
2330	2335	2340
Leu Gln Pro Thr Leu Thr Asn Pro Gly Glu Cys Arg Pro Asn Phe		
2345	2350	2355
Thr Cys Ala Cys Arg Lys Glu Glu Cys Lys Arg Val Ser Pro Pro		
2360	2365	2370
Ser Cys Pro Pro His Arg Leu Pro Thr Leu Arg Lys Thr Gln Cys		
2375	2380	2385
Cys Asp Glu Tyr Glu Cys Ala Cys Asn Cys Val Asn Ser Thr Val		
2390	2395	2400
Ser Cys Pro Leu Gly Tyr Leu Ala Ser Thr Ala Thr Asn Asp Cys		
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Gly Cys Thr Thr Thr Thr Cys Leu Pro Asp Lys Val Cys Val His		
2420	2425	2430
Arg Ser Thr Ile Tyr Pro Val Gly Gln Phe Trp Glu Glu Gly Cys		
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Asp Val Cys Thr Cys Thr Asp Met Glu Asp Ala Val Met Gly Leu		
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Arg Val Ala Gln Cys Ser Gln Lys Pro Cys Glu Asp Ser Cys Arg		
2465	2470	2475
Ser Gly Phe Thr Tyr Val Leu His Glu Gly Glu Cys Cys Gly Arg		

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Cys Leu Pro Ser Ala Cys Glu Val Val Thr Gly Ser Pro Arg Gly				
2495		2500		2505
Asp Ser Gln Ser Ser Trp Lys Ser Val Gly Ser Gln Trp Ala Ser				
2510		2515		2520
Pro Glu Asn Pro Cys Leu Ile Asn Glu Cys Val Arg Val Lys Glu				
2525		2530		2535
Glu Val Phe Ile Gln Gln Arg Asn Val Ser Cys Pro Gln Leu Glu				
2540		2545		2550
Val Pro Val Cys Pro Ser Gly Phe Gln Leu Ser Cys Lys Thr Ser				
2555		2560		2565
Ala Cys Cys Pro Ser Cys Arg Cys Glu Arg Met Glu Ala Cys Met				
2570		2575		2580
Leu Asn Gly Thr Val Ile Gly Pro Gly Lys Thr Val Met Ile Asp				
2585		2590		2595
Val Cys Thr Thr Cys Arg Cys Met Val Gln Val Gly Val Ile Ser				
2600		2605		2610
Gly Phe Lys Leu Glu Cys Arg Lys Thr Thr Cys Asn Pro Cys Pro				
2615		2620		2625
Leu Gly Tyr Lys Glu Glu Asn Asn Thr Gly Glu Cys Cys Gly Arg				
2630		2635		2640
Cys Leu Pro Thr Ala Cys Thr Ile Gln Leu Arg Gly Gly Gln Ile				
2645		2650		2655
Met Thr Leu Lys Arg Asp Glu Thr Leu Gln Asp Gly Cys Asp Thr				
2660		2665		2670
His Phe Cys Lys Val Asn Glu Arg Gly Glu Tyr Phe Trp Glu Lys				
2675		2680		2685
Arg Val Thr Gly Cys Pro Pro Phe Asp Glu His Lys Cys Leu Ala				

2690 2695 2700
 Glu Gly Gly Lys Ile Met Lys Ile Pro Gly Thr Cys Cys Asp Thr
 2705 2710 2715
 Cys Glu Glu Pro Glu Cys Asn Asp Ile Thr Ala Arg Leu Gln Tyr
 2720 2725 2730
 Val Lys Val Gly Ser Cys Lys Ser Glu Val Glu Val Asp Ile His
 2735 2740 2745
 Tyr Cys Gln Gly Lys Cys Ala Ser Lys Ala Met Tyr Ser Ile Asp
 2750 2755 2760
 Ile Asn Asp Val Gln Asp Gln Cys Ser Cys Cys Ser Pro Thr Arg
 2765 2770 2775
 Thr Glu Pro Met Gln Val Ala Leu His Cys Thr Asn Gly Ser Val
 2780 2785 2790
 Val Tyr His Glu Val Leu Asn Ala Met Glu Cys Lys Cys Ser Pro
 2795 2800 2805
 Arg Lys Cys Ser Lys
 2810

<210> 8
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> misc_feature
 <222> (18)..(57)
 <223> n may be any nucleotide (A, T, C or G)

<400> 8
 ggagcgcaçt cagccacnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnttt 60
 cgacctctct gctagc 76

<210> 9

<211> 34
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized primer

 <400> 9
 taatacgact cactatagga gcgcactcag ccac 34

 <210> 10
 <211> 19
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized primer

 <400> 10
 gctagcagag aggtcgaaa 19

 <210> 11
 <211> 76
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <220>
 <221> modified_base
 <222> (1)..(76)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
 are 2'-fluoro

 <400> 11
 ggagcgcacu cagccacaga gccugagug uagaucgcc uagaucuauc gaugcuuuuu 60

 cgaccucucu gcuagc 76

 <210> 12
 <211> 76
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <220>
 <221> modified_base
 <222> (1)..(76)

<223> all purines (A and G) are 2'OH (ribo); all pyrimidines (U and C)
are 2'-fluoro

<400> 12
ggagcgcacu cagccacaac acuaaugggg aaaguucaag gauucuugac cggugcguuu 60
cgaccucucu gcuagc 76

<210> 13
<211> 76
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(76)
<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
are 2'-fluoro

<400> 13
ggagcgcacu cagccacuaa cggugaucu caggacuaaa uagucaaca ggaugcguuu 60
cgaccucucu gcuagc 76

<210> 14
<211> 76
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(76)
<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
are 2'-fluoro

<400> 14
ggagcgcacu cagccacaga gccugagug uaugaucgcc gagaucuauc gaugcuuuuu 60
cgaccucucu gcuagc 76

<210> 15
<211> 76
<212> DNA
<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(76)

<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 15

ggagcgcacu cagccacgcu cgguggggaa auuuuagccu aauggcuac uugugcguuu 60

cgaccucucu gcuagc 76

<210> 16

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(76)

<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 16

ggagcgcacu cagccacggu ggucagucag ugauaugauu aaguucagcu guggcuguuu 60

cgaccucucu gcuagc 76

<210> 17

<211> 75

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(75)

<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 17

ggagcgcacu cagccacacc gaggcuggau aucuacgaga ggaagugcug cuugaauuuc 60

gaccucucug cuagc 75

<210> 18
 <211> 75
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(75)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
 are 2'-fluoro

<400> 18
 ggagcgcacu cagccacacu gaggcuggau aucuacgaga ggaagugcug cuuggauuuc 60
 gaccucucug cuagc 75

<210> 19
 <211> 75
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(75)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
 are 2'-fluoro

<400> 19
 ggagcgcacu cagccacugg uccuuagcua guuguacuag cgacgcguuc aggugguuuc 60
 gaccucucug cuagc 75

<210> 20
 <211> 75
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(75)

<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 20
 ggagcgcacu cagccacuaa cggugaucu caggacuaau agucaacaag gaugcguuuc 60
 gaccucucug cuagc 75

<210> 21
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(75)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 21
 ggagcgcacu cagccacuaa cggcugaucu caggacuaaa uagucaacaa ggaugcguuu 60
 cgaccucucu gcuagc 76

<210> 22
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(75)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 22
 ggagcgcacu cagccacccu gucgucuuuu gguagucagc caaaagcuag uugguuguuu 60
 cgaccucucu gcuagc 76

<210> 23
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(76)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 23
 ggagcgcacu cagccacccu cgcaagcau uuaagaauga cuugugccgc uggcuguuuu 60
 cgaccucucu gcuagc 76

<210> 24
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(76)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 24
 ggagcgcacu cagccacuuu acggugaaag ucucucgggg uuccgaguua cggugcguuu 60
 cgaccucucu gcuagc 76

<210> 25
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<220>
 <221> modified_base
 <222> (1)..(76)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 25
 ggagcgcacu cagccacggu aacauuguuu ccggcgauuc uuugaacgcc gucgugguuu 60

cgaccucucu gcuagc

76

<210> 26

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(76)

<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<400> 26

ggagcgcacu cagccaccag uuaugcuggc uuuggucuuu gacugucuga guguucguuu

60

cgaccucucu gcuagc

76

<210> 27

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(76)

<223> all pyrines (A and G) are 2'-OH; all pyrimidines (U and C) are 2'-fluoro

<400> 27

ggagcgcacu cagccacugg ggcugaucuc gcacgauagu ucgugucaag gaugcguuuu

60

cgaccucucu gcuagc

76

<210> 28

<211> 75

<212> DNA

<213> Artificial

<220>

<223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C) are 2'-fluoro

<220>

<221> modified_base
 <222> (1)..(75)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
 are 2'-fluoro

<400> 28
 ggagcgcacu cagccacgcc cagcucaau uauagucuac uuugaugugc ccgugguuc 60
 gaccucucug cuagc 75

<210> 29
 <211> 73
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(73)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
 are 2'-fluoro

<400> 29
 ggagcgcacu cagccacgcu guacacugau guuguaacau guacccccug gcuguuucga 60
 ccucucugcu agc 73

<210> 30
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> (1)..(76)
 <223> all purines (A and G) are 2'-OH (ribo); all pyrimidines (U and C)
 are 2'-fluoro

<400> 30
 ggagcgcacu cagccacuuc gacuuucaug ucugaagucc cugcagugcg agagacguuu 60
 cgaccucucu gcuagc 76

<210> 31
 <211> 76
 <212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 31

ggagcgcact cagccacagt tctgtcgggtg atgaattagc gcgagagctg tgggacgttt 60

cgacctctct gctagc 76

<210> 32

<211> 75

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 32

ggagcgcact cagccacaaa cggacgggtga tggattaacg cgggtttatg gcaagggttc 60

gacctctctg ctagc 75

<210> 33

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 33

ggagcgcact cagccacggc acgacgggtga tggattagcg cgggtgcgggt ggtgtcattt 60

cgacctctct gctagc 76

<210> 34

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 34

ggagcgcact cagccactca aggggggtcgc gtgggggacga agggttgcag tgtgtcgttt 60

cgacctctct gctagc 76

<210> 35

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 35

ggagcgcaact cagccacggc acgacgggtga tgaattagcg cgggtgtcggg ggtgtcattt 60

cgacctctct gctagc 76

<210> 36

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 36

ggagcgcaact cagccacgga gcgtcgggtga tggattagcg cgggtccgtg gtacacattt 60

cgacctctct gctagc 76

<210> 37

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 37

ggagcgcaact cagccacgga gcgtcgggtga tggattagcg cgggtccgtg gtacaccttt 60

cgacctctct gctagc 76

<210> 38

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 38

ggagcgcaact cagccacggc atgacgggtga tgaattagcg cgggtgtcggg ggtgtcattt 60

cgacctctct gctagc 76

<210> 39

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 39

ggagcgcaact cagccacgga gcgtcgggtga tggattagcg cggctccgtg gtacgccttt 60

cgacctctct gctagc 76

<210> 40

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 40

ggagcgcaact cagccacgga gcgtcgggtga tggattagcg cggctccgtg gtacaccttt 60

cgacctctct gctagc 76

<210> 41

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 41

ggagcgcaact cagccacggc acgacggtga tgaattagcg cgggtgctcgtt ggtgttattt 60

cgacctctct gctagc 76

<210> 42

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 42

ggagcgcaact cagccaccac ggggacgggt agggcgggcg aggtggtggc attagcgttt 60

cgacctctct gctagc 76

<210> 43

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 43

ggagcgcact cagccacagt tctgtcgggtg atgaattagc gcgggagctg tgggacgttt 60

cgacctctct gctagc 76

<210> 44

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 44

ggagcgcact cagccacggg gtgggtagac ggcgggtatg tggctggtgt cgaagggttt 60

cgacctctct gctagc 76

<210> 45

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 45

ggagcgcact cagccacgac ggtgatggat tagcgcgggtg gagaagatgc gctgttgttt 60

cgacctctct gctagc 76

<210> 46

<211> 75

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 46

ggagcgcact cagccacgac ggtgatggat tagcgcgggtg gatcttaacg tgcgagtttc 60

gacctctctg ctagc 75

<210> 47

<211> 77

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 47

ggagcgcact cagccactga agggtaagga cgaggagggt atacagtgtg cgcgtgtatt 60

tcgacctctc tgctagc 77

<210> 48

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 48

ggagcgcact cagccacaac tggttgtcgg tgatggcatt aacgcggacc aggcattgtt 60

cgacctctct gctagc 76

<210> 49

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 49

ggagcgcact cagccacacg acattggcgg gttgtaatta ccacgcatgg ctgtttgttt 60

cgacctctct gctagc 76

<210> 50

<211> 76

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 50

ggagcgcact cagccactgt tgccgacggt gatgtattaa cgcgggcaac gttggtgttt 60

cgacctctct gctagc 76

<210> 51

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> misc_feature

<222> (21)..(60)

<223> n may be any nucleotide (A, T, G, or C)

<400> 51
ctacctacga tctgactagc nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 60

gcttactctc atgtagttcc 80

<210> 52

<211> 20

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 52
ctacctacga tctgactagc 20

<210> 53

<211> 21

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 53
aggaactaca tgagagtaag c 21

<210> 54

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 54
ctacctacga tctgactagc ggaatgagaa tgctgatgga ttgctcaggt ctgctggctg 60

cttactctca tgtagttcc 79

<210> 55

<211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 55
 ctacctacga tctgactagc ggaatgagaa tgctggtgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 56
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 56
 ctacctacga tctgactagc ggaacgagaa tgctgatgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 57
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 57
 ctacctacga tctgactagc ggaataagaa tgctgatgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 58
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 58
 ctacctacga tctgactagc ggaatgagaa tgttgatgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 59

<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 59
ctacctacga tctgactagc ggaatgagag tgctgatgga ttgctcaggt ctgctggctg 60
cttactctca tgtagttcc 79

<210> 60
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 60
ctacctacga tctgactagc ggaatgagaa tgttggtgga ttgctcaggt ctgctggctg 60
cttactctca tgtagttcc 79

<210> 61
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 61
ctacctacga tctgactagc ggaatgagag tgctggtgga ttgctcaggt ctgctggctg 60
cttactctca tgtagttcc 79

<210> 62
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 62
ctacctacga tctgactagc ggaatgagaa tgctgatgga ttgttcaggt ctgctggctg 60
cttactctca tgtagttcc 79

<210> 63

<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 63
ctacctacga tctgactagc ggaatgagaa tgctgatgga ttgctcaggt ctgctgactg 60

cttactctca ttagttcc 79

<210> 64
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 64
ctacctacga tctgactagc ggaatgagaa tgctgatgga ttgccaggt ctgctggctg 60

cttactctca ttagttcc 79

<210> 65
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 65
ctacctacga tctgactagc ggaatgagaa tgctggtgga ttgccaggt ctgctggctg 60

cttactctca ttagttcc 79

<210> 66
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 66
ctacctacga tctgactagc ggaatgagaa tgttggtgga ttgctcaggt ctgctggctg 60

cttactctca ttagttcc 79

<210> 67

<211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 67
 ctacctacga tctgactagc ggaatgagga tgctggtgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 68
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 68
 ctacctacga tctgactagc ggaatgagag tgctgatgga ttgctcaggt ctactggctg 60
 cttactctca tgtagttcc 79

 <210> 69
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 69
 ctacctacga tctgactagc ggaatgagga tgctgatgga ttggtcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 70
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 70
 ctacctacga tctgactagc gggatgagag tgctggtgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 71

<211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemcially synthesized

 <400> 71
 ctacctacga tctgactagc gcaatgagga tgctgatgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 72
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 72
 ctacctacga tctgactagc ggaatgagga tgctgatgga ttgcacaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 73
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 73
 ctacctacga tctgactagc ggaatgagga tgctggtgga ttgctcaggt ctgctggctg 60
 cttactctca tgtagttcc 79

 <210> 74
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 74
 ctacctacga tctgactagc gaaacactag gttgggttagg attggtgtgt ttccgttctg 60
 cttactctca tgtagttcc 79

 <210> 75

<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 75
ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt tcccgctctg 60
cttactctca ttagttcc 79

<210> 76
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 76
ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt tcccgccctg 60
cttactctca ttagttcc 79

<210> 77
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 77
ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt ttctgctctg 60
cttactctca ttagttcc 79

<210> 78
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 78
ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt ttccgctttg 60
cttactctca ttagttcc 79

<210> 79

<211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 79
 ctacctacga tctgactagc ggaacactag gttggtagg attggtgtgt tcccgttttg 60

 cttactctca ttagttcc 79

 <210> 80
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 80
 ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt tcccgttttg 60

 cttactctca ttagttcc 79

 <210> 81
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 81
 ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt tcccgtatg 60

 cttactctca ttagttcc 79

 <210> 82
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 82
 ctacctacga tctgactagc gaaacactag gttggtagg attggtgtgt tcccgtatg 60

 cttactctca ttagttcc 79

 <210> 83

<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 83
ctacctacga tctgactagc tgagtagtta gtaacttttt attatgggtt ggtgggtctg 60
gcttactctc atgtagttcc 80

<210> 84
<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 84
ctacctacga tctgactagc tgagtagtca gtaatttttt attatgggtt ggtgggcctg 60
gcttactctc atgtagttcc 80

<210> 85
<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 85
ctacctacga tctgactagc aaggggattg gctccgggtc tggcgtgctt ggcacctctg 60
gcttactctc atgtagttcc 80

<210> 86
<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 86
ctacctacga tctgactagc aaggggattg gctccgggtc tggcgtgctt ggtacctccg 60
gcttactctc atgtagttcc 80

<210> 87

<211> 80
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 87
 ctacctacga tctgactagc aaggggattg gctccgggtc tggcgtgctt ggcattctcg 60

 gcttactctc atgtagttcc 80

 <210> 88
 <211> 80
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 88
 ctacctacga tctgactagc aaggggattg gctccgggtc tggcgtgctc ggcacctttg 60

 gcttactctc atgtagttcc 80

 <210> 89
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 89
 ctacctacga tctgactagc ggaatgagaa ggctggtgga ttgctcaggt ctgctggctg 60

 cttactctca tgtagttcc 79

 <210> 90
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 90
 ctacctacga tctgactagc ggaatgagta tgctgatgga ttgctcaggt ctgctggctg 60

 cttactctca tgtagttcc 79

 <210> 91

<211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 91
 ctacctacga tctgactagc ggaatgagaa ggctgatgga ttgctcaggt ctgctggctg 60

 cttactctca tgtagttcc 79

 <210> 92
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 92
 ctacctacga tctgactagc ggaatgagag cgctgatgga ttgctcaggt ctgctggctg 60

 cttactctca tgtagttcc 79

 <210> 93
 <211> 80
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 93
 ctacctacga tctgactagc aaggggattg gctccgggctc tggcgtgctc ggcacttccg 60

 gcttactctc atgtagttcc 80

 <210> 94
 <211> 80
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 94
 ctacctacga tctgactagc aaggggattg gctccgggctc tggcgtgctc ggcaccttcc 60

 gcttactctc atgtagttcc 80

 <210> 95

<211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <220>
 <221> misc_feature
 <222> (29)..(57)
 <223> where Y is C or T; R is A or G; H is A, C, or T.

 <400> 95
 ctacctacga tctgactagc ggaatgagra tgytgrtgga ttgchcaggt ctrytgrctg 60
 cttactctca thtagttcc 79

 <210> 96
 <211> 78
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <220>
 <221> misc_feature
 <222> (22)..(58)
 <223> where Y is C or T; R is A or G; H is A, C, or T.

 <400> 96
 ctacctacga tctgactagc graacactag gttggtagg rttggtgtgt tcygyyyhgc 60
 ttactctcat gtagttcc 78

 <210> 97
 <211> 80
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <220>
 <221> misc_feature
 <222> (49)..(59)
 <223> Where Y is C or T; R is A or G; H is A, C, or T.

 <400> 97
 ctacctacga tctgactagc aaggggattg gctccgggtc tggcgtgcty ggyayyyyyg 60

gcttactctc atgtagttcc 80

<210> 98

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 98

ctacctacga tctgactagc tccagtgttt tatccaataa ccgtgcggtg cctccgtgag 60

cttactctca ttagttcc 79

<210> 99

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 99

ctacctacga tctgactagc tccagtgttt catccaataa ccgtgcggtg cctccgtgag 60

cttactctca ttagttcc 79

<210> 100

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 100

ctacctacga tctgactagc tccagtgttt cattcaataa ccgtgcggtg cctccgtgag 60

cttactctca ttagttcc 79

<210> 101

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 101

ctacctacga tctgactagc tccagtgttt catttaataa ccgtgcggtg cctccgtgag 60

cttactctca thtagttcc 79

<210> 102
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 102
ctacctacga tctgactagc tccagtgttt catctaataa ccgtgcggtg cctccgtgag 60
cttactctca thtagttcc 79

<210> 103
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 103
ctacctacga tctgactagc tccagtgttt catccaataa ccgtgcggtg cttccgtgag 60
cttactctca thtagttcc 79

<210> 104
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 104
ctacctacga tctgactagc tccagtgttt tgtctaataa ccgtgcggtg cctccgtgag 60
cttactctca thtagttcc 79

<210> 105
<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 105
ctacctacga tctgactagc tccagtgttt tatataataa ccgtgcggtg cctccgtgat 60

gcttactctc atgtagttcc 80

<210> 106
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 106
ctacctacga tctgactagc tccagtgttt tattcaataa ccgtgcggtg cctccgtgag 60
cttactctca ttagttcc 79

<210> 107
<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 107
ctacctacga tctgactagc tccagtgttt tattcaataa ccgtgcggtg cctccgtgat 60
gcttactctc atgtagttcc 80

<210> 108
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 108
ctacctacga tctgactagc tccagtgttt tattcaataa ccgtgcggtg tctccgtgag 60
cttactctca ttagttcc 79

<210> 109
<211> 79
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 109
ctacctacga tctgactagc tccagtgttt tatctaataa ccgtgcggtg cctccgtgag 60

cttactctca tgtagttcc 79

<210> 110
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 110
 ctacctacga tctgactagc tccagtgttt tatctaataa ccgtgcggtg cctccgtgat 60
 gcttactctc atgtagttcc 80

<210> 111
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 111
 ctacctacga tctgactagc tccagtgttt tatccaacaa ccgtgcggtg cctccgtgag 60
 cttactctca tgtagttcc 79

<210> 112
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 112
 ctacctacga tctgactagc tccagtgttt tatccaataa ccgtgcgggg cctccgtgat 60
 gcttactctc atgtagttcc 80

<210> 113
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 113
 ctacctacga tctgactagc tccagtgttt tatccaataa ccgtgcggtg cctccgtgat 60

gcttactctc atgtagttcc 80

<210> 114

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 114

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cttactctca ttagttcc 79

<210> 115

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 115

ctacctacga tctgactagc gtgcagtgcc catcttaggc cgtgcggtgc ctccgtcacg 60

cttactctca ttagttcc 79

<210> 116

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 116

ctacctacga tctgactagc gtgcagtgcc tatttttaggc cgtgcggtgc ctccgtcacg 60

cttactctca ttagttcc 79

<210> 117

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 117

ctacctacga tctgactagc gtgcagtgcc tatttttaggt cgtgcggggc ctccgtcacg 60

cttactctca tgtagttcc 79

<210> 118
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 118
 ctacctacga tctgactagc gtgcagtgcc tattctaggc cgtgcggtgc ctccgtcacg 60
 cttactctca tgtagttcc 79

<210> 119
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 119
 ctacctacga tctgactagc gtgcagtgcc tattctaggc cgtgcggtgc ctccgtcatg 60
 cttactctca tgtagttcc 79

<210> 120
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 120
 ctacctacga tctgactagc atgcagtgcc cattctaggc cgtgcggtgc ctccgtcatg 60
 cttactctca tgtagttcc 79

<210> 121
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 121
 ctacctacga tctgactagc ttggtagcga ttctgtggag ctgcggtttg gtcgacgtca 60

gcttactctc atgtagttcc 80

<210> 122
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 122
 ctacctacga tctgactagc ttggtagcga ttttgtggag ctgcggtttg gtcgacgtca 60
 gcttactctc atgtagttcc 80

<210> 123
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 123
 ctacctacga tctgactagc ttggtagtga ctttgtggag ctgcggtttg gtcgacgtca 60
 gcttactctc atgtagttcc 80

<210> 124
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 124
 ctacctacga tctgactagc gtgcagtgcc cattccaggc cgtgcgggtat cctccgtcac 60
 gcttactctc atgtagttcc 80

<210> 125
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 125
 ctacctacga tctgactagc gtgcagtgcc tatcccaggc cgtgcgggtag cctccgtcac 60

gcttactctc atgtagttcc 80

<210> 126

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 126

ctacctacga tctgactagc gtgcagtgcc tatctcaggc cgtgcggtat cctccgtcac 60

gcttactctc atgtagttcc 80

<210> 127

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 127

ctacctacga tctgactagc ttggtagcga ctctgtggag ctgcggtttg gtcgacgtca 60

gcttactctc atgtagttcc 80

<210> 128

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 128

ctacctacga tctgactagc ttggtagcga ctctgtggag ctgcggtctg gtcgacgtca 60

gcttactctc atgtagttcc 80

<210> 129

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 129

ctacctacga tctgactagc ttggtagcga ctctgtggag ctgcggtctg gccgacgtca 60

gcttactctc atgtagttcc 80

<210> 130

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 130

ctacctacga tctgactagc ttggtagcga cactgtggag ctgcggtttg gttgacgtca 60

gcttactctc atgtagttcc 80

<210> 131

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 131

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gcttactctc atgtagttcc 80

<210> 132

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 132

ctacctacga tctgactagc ttggtagcga ctccgtggag ctgcggtttg gtcgacgtca 60

gcttactctc atgtagttcc 80

<210> 133

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 133

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gcttactctc atgtagttcc 80

<210> 134
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 134
 ctacctacga tctgactagc ttggtagcga ctttgtggag ctgcggtttg gtcgacgtca 60
 gcttactctc atgtagttcc 80

<210> 135
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 135
 ctacctacga tctgactagc ttggtagcga ctttgtggag ctgcggtttg gtcgacatca 60
 gcttactctc atgtagttcc 80

<210> 136
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 136
 ctacctacga tctgactagc ttggtagcga ctttgtggag atgcggtttg gttgacgtca 60
 gcttactctc atgtagttcc 80

<210> 137
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 137
 ctacctacga tctgactagc ggaatgagaa tggttggtgga ttgctcaggt ctgctggctg 60

cttactctca tgtagttcc 79

<210> 138

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 138

ctacctacga tctgactagc ggaatgagaa tgctggtgga ttgctcaggt ctgctggctg 60

cttactctca tgtagttcc 79

<210> 139

<211> 78

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 139

ctacctacga tctgactagc ggaatgagaa gctggtggat tgctcaggtc tgctggctgc 60

ttactctcat gtagttcc 78

<210> 140

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 140

ctacctacga tctgactagc ggaatgagaa tgctggtgga ttgctcaggt ctgctggctg 60

cttactctca tgtagttcc 79

<210> 141

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 141

ctacctacga tctgactagc ggaatgagaa tgcaggtgga ttgctcaggt ctgctggctg 60

cttactctca thtagttcc 79

<210> 142

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 142

ctacctacga tctgactagc ggaatgagaa tgcagatgga ttgctcaggt ctgctggctg 60

cttactctca thtagttcc 79

<210> 143

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 143

ctacctacga tctgactagc ggaatgagag tgttggtgga ttgctcaggt ctgctggctg 60

cttactctca thtagttcc 79

<210> 144

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 144

ctacctacga tctgactagc ggaatgagag tgctggtgga ttgctcaggt ctgctggctg 60

cttactctca thtagttcc 79

<210> 145

<211> 79

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 145

ctacctacga tctgactagc ggaatgagta tgctggtgga ttgctcaggt ctgctggctg 60

cttactctca ttagttcc 79

<210> 146
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 146
 ctacctacga tctgactagc ggaatgagta tgctgatgga ttgctcaggt ctgctggctg 60
 cttactctca ttagttcc 79

<210> 147
 <211> 78
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 147
 ctacctacga tctgactagc ttgtcgact tttggttggt ctggttggtt ctaagtgcgc 60
 ttactctcat gtagttcc 78

<210> 148
 <211> 78
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 148
 ctacctacga tctgactagc ttgtcgact tttggttggt ctggttggtt ttaagtgcgc 60
 ttactctcat gtagttcc 78

<210> 149
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 149
 ctacctacga tctgactagc ttggtagcga cacagtggag ctgcggtttg gtcgacgtca 60

gcttactctc atgtagttcc 80

<210> 150

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 150

ctacctacga tctgactagc tcaaagtatt acttattggc aataagtcgt ttactctata 60

gcttactctc atgtagttcc 80

<210> 151

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 151

ctacctacga tctgactagc tttcagtctt ccacatttat agggtttggc attgggtctg 60

gcttactctc atgtagttcc 80

<210> 152

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 152

ctacctacga tctgactagc tttcagtctt ctacatttat agggtttggc attgggtctg 60

gcttactctc atgtagttcc 80

<210> 153

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 153

ctacctacga tctgactagc tttcagtctt ccacgtttat agggtttggc attgggtctg 60

gcttactctc atgtagttcc 80

<210> 154
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 154
 ctacctacga tctgactagc ttttagtctt ccacatttat agggtttggc attgggtctg 60
 gcttactctc atgtagttcc 80

<210> 155
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 155
 ctacctacga tctgactagc tttcagtctt tcacatttat agggtttggc attgggtctg 60
 gcttactctc atgtagttcc 80

<210> 156
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 156
 ctacctacga tctgactagc tttcagtctt tcatatttat agggtttggc attgggtctg 60
 gcttactctc atgtagttcc 80

<210> 157
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 157
 ctacctacga tctgactagc cagttctggg aaaaattatt tttttatttc gatcgtatat 60

gcttactctc atgtagttcc 80

<210> 158

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 158

ctacctacga tctgactagc cagttctggg aaaaattatt tttttatttc gatcgtattt 60

gcttactctc atgtagttcc 80

<210> 159

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 159

ctacctacga tctgactagc ctcagattga ctccggccga cttgttttaa tcttctgagt 60

gcttactctc atgtagttcc 80

<210> 160

<211> 80

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 160

ctacctacga tctgactagc cccacttat cgtgtacctt atgatatgtc gaatactctt 60

gcttactctc atgtagttcc 80

<210> 161

<211> 78

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 161

ctacctacga tctgactagc cttacctatt cccttctgcg gaatacgtcg agtactatgc 60

ttactctcat gtagttcc 78

<210> 162
 <211> 79
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 162
 ctacctacga tctgactagc cagttctggg aaaaatcatt ttttatttcg atcgtatttg 60
 cttactctca ttagttcc 79

<210> 163
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 163
 ctacctacga tctgactagc aaggggattg gctccgggtc tggcgtgctt ggcattcttg 60
 gcttactctc atgtagttcc 80

<210> 164
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 164
 ctacctacga tctgactagc ctcagattga ctccgggtga cttgttttaa tcttctgagt 60
 gcttactctc atgtagttcc 80

<210> 165
 <211> 56
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 165
 ggagcgcact cagccaccct cgcaagcatt ttaagaatga cttgtgccgc tggctg 56

<210> 166
 <211> 25
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized primer

 <400> 166
 gatcgatcta atacgactca ctata 25

 <210> 167
 <211> 18
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized primer

 <400> 167
 cagccagcgg cacaagtc 18

 <210> 168
 <211> 79
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 168
 tcgatcta at acgactcact ataggagcgc actcagccac cctcgcaagc attttaagaa 60
 tgacttggtgc cgctggctg 79

 <210> 169
 <211> 46
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 169
 ggaccaccct cgcaagcatt ttaagaatga cttgtgccgc tgggcc 46

 <210> 170
 <211> 18
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 170
 ggaccagcgg cacaagtc 18

 <210> 171
 <211> 71
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 171
 gatcgatcta atacgactca ctataggacc accctcgcaa gcattttaag aatgacttgt 60
 gccgctggtc c 71

 <210> 172
 <211> 45
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 172
 ggaccaccct cgcaagcatt gagaaatgac ttgtgccgct ggtcc 45

 <210> 173
 <211> 70
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 173
 gatcgatcta atacgactca ctataggacc accctcgcaa gcattgagaa atgacttgtg 60
 ccgctgggtcc 70

 <210> 174
 <211> 36
 <212> DNA
 <213> Artificial

 <220>
 <223> Description of Artificial Sequence: chemically synthesized

 <400> 174

ggaccaccct cgcaacgaga gttgtgccgc tggccc

36

<210> 175

<211> 18

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 175

ggaccagcgg cacaactc

18

<210> 176

<211> 61

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 176

gacgatcta atacgactca ctataggacc accctcgcaa cgagagttgt gccgctggtc

60

c

61

<210> 177

<211> 45

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 177

ggagcgcaact cagccacggg gtgggtagac ggcgggtatg tggct

45

<210> 178

<211> 18

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 178

agccacatac ccgccgtc

18

<210> 179

<211> 70

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 179

gatcgatcta atacgactca ctataggagc gcactcagcc acgggggtggg tagacggcgg 60

gtatgtggct 70

<210> 180

<211> 38

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 180

ggagccacgg ggtgggtaga cggcgggtat gtggctcc 38

<210> 181

<211> 18

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 181

ggagccacat acccgccg 18

<210> 182

<211> 63

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 182

gatcgatcta atacgactca ctataggagc cacgggggtgg gtagacggcg ggtatgtggc 60

tcc 63

<210> 183

<211> 32

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 183
gggacgggggt gggtagacgg cgggtatgtc cc 32

<210> 184
<211> 15
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized primer

<400> 184
gggacatacc cgccg 15

<210> 185
<211> 57
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 185
gatcgatcta atacgactca ctatagggac ggggtgggta gacggcgggt atgtccc 57

<210> 186
<211> 53
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 186
ggagcgcact cagccacacg acattggcgg gttgtaatta ccacgcatgg ctg 53

<210> 187
<211> 18
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized primer

<400> 187
cagccatgcg tggtaatt 18

<210> 188
<211> 78
<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 188

gatcgatcta atacgactca ctataggagc gcactcagcc acacgacatt ggcggggttg 60

aattaccacg catggctg 78

<210> 189

<211> 45

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 189

ggagccacac gacattggcg ggttgtaatt accacgcatg gctcc 45

<210> 190

<211> 14

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 190

ggagccatgc gtgg 14

<210> 191

<211> 70

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 191

gatcgatcta atacgactca ctataggagc cacacgacat tggcggggttg taattaccac 60

gcatggctcc 70

<210> 192

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 192
ggagccacac gacattggcg ggcgagagcc acgcatggct cc 42

<210> 193
<211> 67
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 193
gatcgatcta atacgactca ctataggagc cacacgacat tggcgggcca gagccacgca 60
tggctcc 67

<210> 194
<211> 38
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 194
ggagccacac gacattggcg agagccacgc atggctcc 38

<210> 195
<211> 63
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 195
gatcgatcta atacgactca ctataggagc cacacgacat tggcgagagc cacgcatggc 60
tcc 63

<210> 196
<211> 45
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 196
ggagccacac gagagtggcg ggttgtaatt accacgcatg gctcc 45

<210> 197
 <211> 70
 <212> DNA
 <213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 197
 gatcgatcta atacgactca ctataggagc cacacgagag tggcgggttg taattaccac 60
 gcatggctcc 70

<210> 198
 <211> 38
 <212> DNA
 <213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 198
 ggccacacga cattggcggg cgagagccac gcatggcc 38

<210> 199
 <211> 17
 <212> DNA
 <213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 199
 ggccatgcgt ggctctc 17

<210> 200
 <211> 63
 <212> DNA
 <213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 200
 gatcgatcta atacgactca ctataggcca cagcagattg gcgggcgaga gccacgcatg 60
 gcc 63

<210> 201
 <211> 37
 <212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 201

ggagccacac gacattggcg cgagagcgca tggctcc

37

<210> 202

<211> 17

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized primer

<400> 202

ggagccatgc gctctcg

17

<210> 203

<211> 62

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 203

gatcgatcta atacgactca ctataggagc cacacgacat tggcgcgaga gcgcatggct

60

cc

62

<210> 204

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 204

ccagcggaat gagaatgctg atggattgct caggtctgct gg

42

<210> 205

<211> 48

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 205

atgagagtgc tggaggattg ctcaggtctg ctggctgctt actctcat 48

<210> 206

<211> 36

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 206

cgatctgact agcggaatga gaatgctggt ggatcg 36

<210> 207

<211> 44

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 207

gatctgacta gcgcaatgag gatgctgat ggattgctca ggtc 44

<210> 208

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 208

ggacgatctg actagctcca gtgttttatc taataaccgt cc 42

<210> 209

<211> 49

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<400> 209

ggagctccag tgttttatct aataaccgtg cggtgccctcc gtgagctcc 49

<210> 210

<211> 29

<212> DNA

<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 210
ggagctgcgg tttggtcgac gtcagctcc 29

<210> 211
<211> 29
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 211
ggtagcgact ctgtggagct gcggtttgg 29

<210> 212
<211> 46
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature
<222> (46)..(46)
<223> thymidine at position 46 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 212
ggcgtgcagt gcctattcta ggccgtgcgg tgccctccgtc acgcct 46

<210> 213
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature
<222> (13)..(14)
<223> thymidine at position 13 is modified by a PEG and attached to the adenosine at position 14

<220>
<221> misc_feature
<222> (40)..(40)

<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 213
gcgtgcagtg cctaggccgt gcggtgcctc cgtcacgcct 40

<210> 214
<211> 39
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature
<222> (13)..(14)
<223> cytidine at position 13 is modified by a PEG and attached to the the guanosine at position 14

<220>
<221> misc_feature
<222> (39)..(39)
<223> thymidine at position 39 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 214
ggcgtgcagtg gccggccgtg cgggtgcctcc gtcacgcct 39

<210> 215
<211> 41
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature
<222> (30)..(31)
<223> guanosine at position 30 is modified by a PEG and attached to the cytidine at position 31

<220>
<221> misc_feature
<222> (41)..(41)
<223> thymidine at position 41 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 215
ggcgtgcagtg gcctattcta ggccgtgcgg ccgtcacgcc t 41

<210> 216
<211> 36
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature
<222> (14)..(15)
<223> thymidine at position 14 is modified by a PEG and attached to the
adenosine at position 15

<220>
<221> misc_feature
<222> (25)..(26)
<223> guanosine at position 25 is modified by a PEG and attached to the
cytidine at position 26

<220>
<221> misc_feature
<222> (36)..(36)
<223> thymidine at position 36 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 216
ggcgtgcagt gcctaggccg tgcggccgtc acgcct 36

<210> 217
<211> 36
<212> DNA
<213> Artificial

<220>
<223> chemically synthesized

<220>
<221> misc_feature
<222> (1)..(4)
<223> n is a, t, c or g

<220>
<221> misc_feature
<222> (15)..(16)
<223> n is a, t, c or g

<220>
<221> misc_feature
<222> (17)..(20)
<223> n is a, t, c or g and represents 1 to 4 nucleotides

<220>
<221> misc_feature
<222> (21)..(22)
<223> n is a, t, c or g

<220>
<221> misc_feature
<222> (33)..(36)
<223> n is a, t, c or g

<400> 217
nnnnccacct cgcannnnnn nntgtgccgc tgnnnn

36

<210> 218
<211> 32
<212> DNA
<213> Artificial

<220>
<223> chemically synthesized

<220>
<221> misc_feature
<222> (1)..(5)
<223> n is a, t, c or g

<220>
<221> misc_feature
<222> (28)..(32)
<223> n is a, t, c or g

<400> 218
nnnnnrggry rggtagacgg cgggyrtnnn nn

32

<210> 219
<211> 38
<212> DNA
<213> Artificial

<220>
<223> chemically synthesized

<220>
<221> misc_feature
<222> (1)..(2)
<223> n is a, t, c or g

<220>
<221> misc_feature
<222> (21)..(26)
<223> n is a, t, c or g

<220>
<221> misc_feature
<222> (37)..(38)
<223> n is a, t, c or g

<400> 219
nnccacacga cattggcggg nnnnnnccac gcatggnn 38

<210> 220
<211> 37
<212> DNA
<213> Artificial

<220>
<223> chemically synthesized

<220>
<221> misc_feature
<222> (1)..(5)
<223> n is t, c, g or a

<220>
<221> misc_feature
<222> (11)..(12)
<223> n is t, c, g or a

<220>
<221> misc_feature
<222> (13)..(13)
<223> n is t, c, g or a and represents 3 to 10 nucleotides

<220>
<221> misc_feature
<222> (14)..(15)
<223> n is t, c, g or a

<220>
<221> misc_feature
<222> (33)..(37)
<223> n is t, c, g or a

<400> 220
nnnnncagtg nnnnnycgtg cgkryytcc gtannnn 37

<210> 221
<211> 41
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 221

ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc c

41

<210> 222

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 222

ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct

42

<210> 223

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(1)

<223> guanosine at position 1 is 2'-O-Methyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 223

ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct

42

<210> 224

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base
<222> (2)..(2)
<223> guanosine at position 2 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 224
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 225
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (3)..(3)
<223> cytidine at position 3 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 225
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 226
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (4)..(4)
<223> guanosine at position 4 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to

3' linked)

<400> 226
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 227
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (5)..(5)
<223> uracil at position 5 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 227
ggcgugcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 228
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (6)..(6)
<223> guanosine at position 6 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 228
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 229
<211> 42

<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (7)..(7)
<223> cytidine at position 7 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 229
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 230
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (8)..(8)
<223> adenosine at position 8 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 230
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 231
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base
<222> (9)..(9)
<223> guanosine at position 9 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 231
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 232
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (10)..(10)
<223> Uracil at position 10 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 232
ggcgtgcagu gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 233
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (11)..(11)
<223> guanosine at position 11 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to

3' linked)

<400> 233
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 234
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (12)..(12)
<223> cytidine at position 12 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 234
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 235
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (13)..(13)
<223> cytidine at position 13 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 235
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 236
<211> 42

<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (14)..(14)
<223> uracil at position 14 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 236
ggcgtgcagt gccutcgcc gtgcggtgcc tccgtcacgc ct 42

<210> 237
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (15)..(15)
<223> uracil at position 15 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 237
ggcgtgcagt gcctucggcc gtgcggtgcc tccgtcacgc ct 42

<210> 238
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base
<222> (16)..(16)
<223> cytidine at position 16 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 238
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 239
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (17)..(17)
<223> guanosine at position 17 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 239
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 240
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (18)..(18)
<223> guanosine at position 18 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to

3' linked)

<400> 240
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 241
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (19)..(19)
<223> Cytidine at position 19 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 241
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 242
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (20)..(20)
<223> cyidine at position 20 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 242
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 243
<211> 42

<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (21)..(21)
<223> guanosine at position 21 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 243
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 244
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (22)..(22)
<223> uracil at position 22 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 244
ggcgtgcagt gccttcggcc gugcggtgcc tccgtcacgc ct 42

<210> 245
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base
<222> (23)..(23)
<223> guanosine at position 23 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 245
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 246
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (24)..(24)
<223> cytidine at position 24 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 246
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 247
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (25)..(25)
<223> guanosine at position 25 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to

3' linked)

<400> 247
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 248
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (26)..(26)
<223> guanosine at position 26 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' tp
3' linked)

<400> 248
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 249
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (27)..(27)
<223> uracil at position 27 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 249
ggcgtgcagt gccttcggcc gtgcggugcc tccgtcacgc ct 42

<210> 250
<211> 42

<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (28)..(28)
<223> guanosine at position 28 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 250
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 251
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (29)..(29)
<223> cytidine at position 29 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 251
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 252
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base
<222> (30)..(30)
<223> cytidine at position 30 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine

<400> 252
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 253
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (31)..(31)
<223> uracil at position 31 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 253
ggcgtgcagt gccttcggcc gtgcggtgcc uccgtcacgc ct 42

<210> 254
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (32)..(32)
<223> cytidine at position 32 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 254
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 255
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (33)..(33)
<223> Cytidine at position 33 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 255
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 256
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (34)..(34)
<223> guanosine at position 34 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 256
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 257
<211> 42
<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (35)..(35)

<223> uracil at position 35 is 2'-O-Methyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 257

ggcgtgcagt gccttcggcc gtgcggtgcc tccgucacgc ct 42

<210> 258

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (36)..(36)

<223> cytidine at position 36 is 2'-OMethyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine

<400> 258

ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 259

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (37)..(37)

<223> adenosine at position 37 is 2'-OMethyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 259

ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct

42

<210> 260

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (38)..(38)

<223> cytidine at position 38 is 2'-O-Methyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 260

ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct

42

<210> 261

<211> 42

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (39)..(39)

<223> guanosine at position 39 is 2'-O-Methyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 261
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 262
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (40)..(40)
<223> cytidine at position 40 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 262
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 263
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (41)..(41)
<223> cytidine at position 41 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 263
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 264
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(2)
<223> guanosines at positions 1 and 2 are 2'-O-Methyl

<220>
<221> modified_base
<222> (4)..(4)
<223> guanosine at position 4 is 2'-O-Methyl

<220>
<221> modified_base
<222> (6)..(6)
<223> guanosine at position 6 is 2'-O-Methyl

<220>
<221> modified_base
<222> (37)..(37)
<223> adenosine at position 37 is 2'-O-Methyl

<220>
<221> modified_base
<222> (39)..(39)
<223> guanosine at position 37 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 264
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 265
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (3)..(3)
<223> cytidine at position 3 is 2'-O-Methyl

<220>
<221> modified_base

<222> (5)..(5)
<223> uracil at position 5 is 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(36)
<223> cytidine at position 36 is 2'-O-Methyl

<220>
<221> modified_base
<222> (38)..(38)
<223> cytidine at position 3 is 2'-O-Methyl

<220>
<221> modified_base
<222> (40)..(40)
<223> cytidine at position 36 is 2'-O-Methyl

<220>
<221> modified_base
<222> (41)..(41)
<223> cytidine at position 36 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 265
ggcgugcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 266
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(2)
<223> guanosines at positions 1 and 2 are 2'-O-Methyl

<220>
<221> modified_base
<222> (3)..(3)
<223> cytidine at position 3 is 2'-O-Methyl

<220>
<221> modified_base
<222> (4)..(4)
<223> guanosine at position 4 is 2'-O-Methyl

<220>
<221> modified_base
<222> (5)..(5)
<223> uracil at position 5 is 2'-O-Methyl

<220>
<221> modified_base
<222> (6)..(6)
<223> guanosine at position 6 is 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(36)
<223> cytidine at position 36 is 2'-O-Methyl

<220>
<221> modified_base
<222> (37)..(37)
<223> adenosine at position 37 is 2'-O-Methyl

<220>
<221> modified_base
<222> (38)..(38)
<223> cytidine at position 38 is 2'-O-Methyl

<220>
<221> modified_base
<222> (39)..(39)
<223> guanosine at position 39 is 2'-O-Methyl

<220>
<221> modified_base
<222> (40)..(41)
<223> cytidines at positions 40 and 41 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 266
ggcgugcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 267
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base
<222> (11)..(11)
<223> guanosine at position 11 is 2'-O-Methyl

<220>
<221> modified_base
<222> (17)..(18)
<223> guanosines at positions 17 and 18 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 267
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 268
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (12)..(13)
<223> cytidines at positions 12 and 13 are 2'-O-Methyl

<220>
<221> modified_base
<222> (19)..(19)
<223> cytidine at position 19 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 268
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 269
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (11)..(11)
<223> guanosine at position 11 is 2'-O-Methyl

<220>
<221> modified_base
<222> (12)..(13)
<223> cytidines at positions 12 and 13 are 2'-O-Methyl

<220>
<221> modified_base
<222> (17)..(18)
<223> guanosines at positions 17 and 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (19)..(19)
<223> cytidine at position 19 is 2'-O-Methyl

<220>
<221> modified_base
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 269
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 270
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (25)..(26)
<223> guanosines at positions 25 and 26 are 2'-O-Methyl

<220>
<221> modified_base
<222> (34)..(34)
<223> guanosine at position 34 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 270
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 271
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (24)..(24)
<223> cytidine at position 24 is 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(33)
<223> cytidines at positions 32 and 33 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 271
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 272
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (24)..(24)
<223> cytidine at position 24 is 2'-O-Methyl

<220>
<221> modified_base
<222> (25)..(26)
<223> guanosines at positions 25 and 26 are 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(33)
<223> cytidines at positions 32 and 33 are 2'-O-Methyl

<220>
<221> modified_base
<222> (34)..(34)
<223> guanosine at position 34 is 2'-O-Methyl

<220>
<221> modified_base
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 272
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 273
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(5)
<223> all residues at positions 1 to 5 are 2'-O Methyl

<220>
<221> modified_base
<222> (37)..(41)
<223> all residues at positions 37 to 41 are 2'-O Methyl

<220>
<221> modified_base
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 273
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 274
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base

<222> (9)..(10)
<223> all residues at positions 9 to 10 are 2'-O-Methyl

<220>
<221> modified_base
<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (23)..(23)
<223> the residue at position 23 is 2'-O-Methyl

<220>
<221> modified_base
<222> (34)..(36)
<223> all residues at positions 34 to 36 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 274
ggcgtgcagu gccttcggcc gtgcggtgcc tccgucacgc ct 42

<210> 275
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (23)..(23)
<223> the residue at position 23 is 2'-O-Methyl

<220>
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<222> (28)..(29)
<223> all residues at positions 28 to 29 are 2'-O-Methyl

<220>
<221> modified_base
<222> (31)..(31)
<223> the residue at position 31 is 2'-O-Methyl

<220>
<221> modified_base
<222> (34)..(36)
<223> all residues at positions 34 to 36 are 2'-O-Methyl

<220>
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<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 275
ggcgtgcagt gccttcggcc gtgcggtgcc uccgucacgc ct 42

<210> 276
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
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<222> (1)..(5)
<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
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<222> (9)..(19)
<223> all residues at positions 9 to 19 are 2'-O-Methyl

<220>
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<222> (37)..(41)
<223> all residues at positions 37 to 41 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 276
ggcgugcagu gccuucggcc gtgcggtgcc tccgtcacgc ct 42

<210> 277
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
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<222> (1)..(5)
<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
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<222> (9)..(19)
<223> all residues at positions 9 to 19 are 2'-O-Methyl

<220>
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<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (23)..(23)
<223> the residue at position 23 is 2'-O-Methyl

<220>
<221> modified_base
<222> (28)..(29)
<223> all residues at positions 28 to 29 are 2'-O-Methyl

<220>
<221> modified_base
<222> (31)..(31)
<223> the residue at position 31 is 2'-O-Methyl

<220>
<221> modified_base
<222> (34)..(41)
<223> all residues at positions 34 to 41 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 277
ggcgugcagu gccuucggcc gtgcggtgcc uccgucacgc ct 42

<210> 278
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (6)..(7)
<223> guanosine at position 6 is modified by a phosphorothioate and attached to the cytidine at position 7

<220>
<221> modified_base
<222> (9)..(19)
<223> all residues at positions 9 to 19 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (19)..(20)
<223> cytidine at position 19 is modified by a phosphorothioate and attached to the cytidine at position 20

<220>
<221> modified_base
<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (23)..(23)
<223> the residue at position 23 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (25)..(26)
<223> guanosine at position 25 is modified by a phosphorothioate and attached to the guanosine at position 26

<220>
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<222> (28)..(29)
<223> all residues at positions 28 to 29 are 2'-O-Methyl

<220>
<221> modified_base
<222> (31)..(31)
<223> the residue at position 31 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (32)..(33)
<223> cytidine at position 32 is modified by a phosphorothioate and attached to the cytidine at position 33

<220>
<221> modified_base
<222> (34)..(41)
<223> all residues at positions 34 to 41 are 2'-O-Methyl

<220>

<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 278
ggcgugcagu gccuucggcc gtgcggtgcc uccgucacgc ct 42

<210> 279
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
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<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
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<222> (6)..(7)
<223> guanosine at position 6 is modified by a phosphorothioate and attached to the cytidine at position 7

<220>
<221> misc_feature
<222> (7)..(8)
<223> cytidine at position 7 is modified by a phosphorothioate and attached to the adenosine at position 8

<220>
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<222> (9)..(19)
<223> all residues at positions 9 to 19 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (19)..(20)
<223> cytidine at position 19 is modified by a phosphorothioate and attached to the cytidine at position 20

<220>
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<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (23)..(23)
<223> the residue at position 23 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (24)..(25)
<223> cytidine at position 24 is modified by a phosphorothioate and attached to the guanosine at position 25

<220>
<221> misc_feature
<222> (25)..(26)
<223> guanosine at position 25 is modified by a phosphorothioate and attached to the guanosine at position 26

<220>
<221> modified_base
<222> (28)..(29)
<223> all residues at positions 28 to 29 are 2'-O-Methyl

<220>
<221> modified_base
<222> (31)..(31)
<223> the residue at position 31 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (32)..(33)
<223> cytidine at position 32 is modified by a phosphorothioate and attached to the cytidine at position 33

<220>
<221> modified_base
<222> (34)..(41)
<223> all residues at positions 34 to 41 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 279
ggcgugcagu gccuucggcc gtgcggtgcc uccgucacgc ct 42

<210> 280
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
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<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (5)..(6)
<223> uracil at position 5 is modified by a phosphorothioate and attached to the guanosine at position 6

<220>
<221> misc_feature
<222> (6)..(7)
<223> guanosine at position 6 is modified by a phosphorothioate and attached to the cytidine at position 7

<220>
<221> misc_feature
<222> (7)..(8)
<223> cytidine at position 7 is modified by a phosphorothioate and attached to the adenosine at position 8

<220>
<221> modified_base
<222> (9)..(19)
<223> all residues at positions 9 to 19 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (19)..(20)
<223> cytidine at position 19 is modified by a phosphorothioate and attached to the cytidine at position 20

<220>
<221> modified_base
<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (21)..(22)
<223> guanosine at position 21 is modified with a phosphorothioate and attached to the thymidine at position 22

<220>
<221> modified_base
<222> (23)..(23)
<223> the residue at position 23 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (23)..(24)
<223> guanosine at position 23 is modified by a phosphorothioate and attached to the cytidine at position 24

<220>
<221> misc_feature
<222> (24)..(25)
<223> cytidine at position 24 is modified by a phosphorothiate and

attached to the guanosine at position 25

<220>

<221> misc_feature

<222> (25)..(26)

<223> guanosine at position 25 is modified by a phosphorothioate and attached to the guanosine at position 26

<220>

<221> misc_feature

<222> (26)..(27)

<223> guanosine at position 26 is modified by a phosphorothioate and attached to the thymidine at position 27

<220>

<221> modified_base

<222> (28)..(29)

<223> all residues at position 28 to 29 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (29)..(30)

<223> cytidine at position 29 is modified by a phosphorothioate and attached to the cytidine at position 30

<220>

<221> modified_base

<222> (31)..(31)

<223> the residue at position 31 is 2'-O-Methyl

<220>

<221> misc_feature

<222> (31)..(32)

<223> uracil at position 31 is modified by a phosphorothioate and attached to the cytidine at position 32

<220>

<221> misc_feature

<222> (32)..(33)

<223> cytidine at position 32 is modified by a phosphorothioate and attached to the cytidine at position 33

<220>

<221> modified_base

<222> (34)..(41)

<223> all residues at positions 34 to 41 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (42)..(42)

<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 280

ggcgugcagu gccuucggcc gtgcggtgcc uccgucacgc ct

42

<210> 281
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(39)
<223> all residues at positions 33 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 281
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct

40

<210> 282

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<211> 40
<212> DNA
<213> Artificial

<220>
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<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
<221> modified_base
<222> (9)..(17)
<223> all residues at positions 9 to 17 are 2'-O-Methyl

<220>
<221> modified_base
<222> (19)..(19)
<223> the residue at position 19 is 2'-O-Methyl

<220>
<221> modified_base
<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (26)..(27)
<223> all residues at positions 26 to 27 are 2'-O-Methyl

<220>
<221> modified_base
<222> (29)..(29)
<223> the residue at position 29 is 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(39)
<223> all residues at positions 32 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to
      3' linked)

<400> 282
ggcgugcagu gcuucgccgt gcggtgccuc cgucacgcct
40

<210> 283
<211> 38
<212> DNA

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<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>

<221> modified_base

<222> (8)..(16)

<223> all residues at positions 8 to 16 are 2'-O-Methyl

<220>

<221> modified_base

<222> (18)..(18)

<223> the residue at position 18 is 2'-O-Methyl

<220>

<221> modified_base

<222> (20)..(20)

<223> the residue at position 20 is 2'-O-Methyl

<220>

<221> modified_base

<222> (25)..(26)

<223> all residues at position 25 to 26 are 2'-O-Methyl

<220>

<221> modified_base

<222> (31)..(37)

<223> all residues at positions 31 to 37 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (38)..(38)

<223> thymidine at position 38 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 283

gcgugcagug cuucgccgtg cgggtgccucc gucacgct

38

<210> 284

<211> 40

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all the residues at postions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all the residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all the residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 284
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<210> 285
<211> 40
<212> DNA
<213> Artificial

<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
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<222> (4)..(5)
<223> uracil at position 4 is modified with a phosphorothioate and attached to the guanosine at position 5

<220>
<221> modified_base
<222> (7)..(18)
<223> all residues at positions 7 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all the residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all of the residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all of the residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 285
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct

<210> 286
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (5)..(6)
<223> guanosine at position 5 is modified by a phosphorothioate and attached to the cytidine at the 6 position

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all the residues at positions 27-28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all the residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 286
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 287
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (6)..(7)
<223> cytidine at position 6 is modified by a phosphorothioate and attached to the adenosine at position 7

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 287
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 288
<211> 40
<212> DNA
<213> Artificial

<220>
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<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (7)..(8)
<223> adenosine at position 7 is modified by a phosphorothioate and attached to the guanosine at position 8

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>

<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 288
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 289
<211> 40
<212> DNA
<213> Artificial

<220>
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<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (18)..(19)
<223> cytidine at position 18 is modified by a phosphorothioate attached to the cytidine at position 19

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base

<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all the residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(38)
<223> all the residues at positions 36 to 38 are 2'-O-Methyl

<220>
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<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 289
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 290
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
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<222> (7)..(18)
<223> all residues at positions 7 to 18 are 2'-O-Methyl

<220>
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<222> (19)..(20)
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attached to the guanosine at the 20 position

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 290
gcgugcagug ccuucggccg tgcggtgccu cgcucacgct 40

<210> 291
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
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<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (20)..(21)
<223> guanosine at position 20 is modified by a phosphorothioate and
attached to the thymidine at position 21

<220>
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<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
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<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
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<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 291
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 292
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (21)..(22)
<223> Thymidine at position 21 is modified by a phosphorothioate and attached to the guanosine at position 22

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
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<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 292
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 293
<211> 40
<212> DNA
<213> Artificial

<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (22)..(23)
<223> guanosine at position 22 is modified by a phosphorothioate and attached to the cytidine at position 23

<220>
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<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base

<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 293
gcgugcagug ccuucggcgcg tgcggtgccu ccgucacgct 40

<210> 294
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (23)..(24)
<223> cytidine at position 23 is modified by a phosphorothioate and attached to the guanosine at position 24

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)

<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

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gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 295
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (24)..(25)
<223> guanosine at position 24 is modified by a phosphorothioate and attached to the guanosine at position 25

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is 3' inverted deoxythymidine (3' to 3' linked)

<400> 295
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 296
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>

<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (25)..(26)
<223> guanosine at position 25 is modified by phosphorothioate and attached to the guanosine at position 26

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 296
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 297
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base

<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (26)..(27)
<223> thymidine at position 26 is modified by phosphorothioate and attached to the guanosine at position 27

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 297
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 298
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (28)..(29)
<223> cytidine at position 28 is modified by a phosphorothioate and
attached to the cytidine at position 29

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at position 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at position 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 298
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct

<210> 299
<211> 40
<212> DNA
<213> Artificial

<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (29)..(30)
<223> cytidine at position 29 is modified by a phosphorothioate and attached to the uracil at position 30

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is 3' inverted deoxythymidine (3' to 3' linked)

<400> 299
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 300
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (30)..(31)
<223> uracil at position 30 is modified by a phosphorothioate and attached to the cytidine at position 31

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is 3' inverted deoxythymidine (3' to 3' linked)

<400> 300
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 301
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base

<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (31)..(32)
<223> cytidine at position 31 is modified by a phosphothioate and attached to the cytidine at position 32

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 301
gcgugcagug ccuucggccg tgcggtgccu ccgucacgt 40

<210> 302
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)

<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (32)..(33)
<223> cytidine at position 32 is modified by a phosphorothioate and attached to the guanosine at position 33

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 302
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 303
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

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<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (34)..(35)
<223> uracil at position 34 is modified by a phosphorothioate and attached to the cytidine at position 35

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 303
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 304
<211> 40
<212> DNA
<213> Artificial

<220>
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<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (35)..(36)
<223> cytidine at position 35 is modified by a phosphorothioate and
attached to the adenosine at position 36

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 304
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct

<210> 305
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (20)..(21)
<223> guanosine at position 20 is modified by a phosphorothioate and attached to the thymidine at position 21

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(39)
<223> all residues at positions 33 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 305
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 306
<211> 40
<212> DNA
<213> Artificial

<220>
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<220>
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<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
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<223> all residues at positions 20 to 22 are 2'-O-Methyl

<220>
<221> modified_base
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<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(39)
<223> all residues at positions 33 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 306
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 307
<211> 40
<212> DNA

<213> Artificial

<220>
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<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (20)..(21)
<223> guanosine at position 20 is modified by a phosphorothioate and attached to the thymidine at position 21

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(39)
<223> all the residues at positions 33 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 307
gcgugcagug ccuuuggccg tgcggtgccu ccgucacgct

<210> 308
<211> 37
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(12)
<223> all residues at positions 8 to 12 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (12)..(13)
<223> cytidine at position 12 is modified by a PEG and attached to the
guanosine at position 13

<220>
<221> modified_base
<222> (13)..(15)
<223> all residues at positions 13 to 15 are 2'-O-Methyl

<220>
<221> modified_base
<222> (17)..(17)
<223> the residue at position 17 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (17)..(18)
<223> guanosine at position 17 is modified by a phosphorothioate and
attached to the thymidine at position 18

<220>
<221> modified_base
<222> (19)..(19)
<223> the residue at position 19 is 2'-O-Methyl

<220>
<221> modified_base
<222> (24)..(25)
<223> all residues at positions 24 and 25 are 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(27)
<223> the residue at position 27 is 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(31)
<223> all residues at positions 30 to 31 are 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(36)
<223> all residues at positions 33 to 36 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (37)..(37)
<223> thymidine at position 37 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 308
gcgugcagug ccggccgtgc ggtgccuccg ucacgct 37

<210> 309
<211> 37
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(12)
<223> all residues at positions 8 to 12 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (12)..(13)
<223> cytidine at position 12 is modified by a PEG and attached to the guanosine at position 13

<220>
<221> modified_base
<222> (13)..(15)
<223> all residues at positions 13 to 15 are 2'-O-Methyl

<220>
<221> modified_base
<222> (17)..(17)
<223> the residue at position 17 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (17)..(18)
<223> guanosine at position 17 is modified with a phosphorothioate and attached to the thymidine at position 18

<220>
<221> modified_base
<222> (19)..(19)
<223> the residue at position 19 is 2'-O-Methyl

<220>
<221> modified_base
<222> (24)..(25)
<223> all residues at positions 24 and 25 are 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(27)
<223> the residue at position 27 is 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(36)
<223> all residues at positions 30 to 36 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (37)..(37)
<223> thymidine at position 37 is 3' inverted deoxythymidine (3' to 3' linked)

<400> 309
gcgugcagug ccggccgtgc ggtgccuccg ucacgct 37

<210> 310
<211> 38
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(3)
<223> all residues at positions 1 to 3 are 2'-O-Methyl

<220>
<221> modified_base
<222> (7)..(17)
<223> all residues at positions 7 to 17 are 2'-O-Methyl

<220>

<221> misc_feature
<222> (19)..(20)
<223> guanosine at position 19 is modified by phosphorothioate and attached to the thymidine at position 20

<220>
<221> modified_base
<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (26)..(27)
<223> all residues at positions 26 to 27 are 2'-O-Methyl

<220>
<221> modified_base
<222> (29)..(29)
<223> the residue at position 29 is 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(37)
<223> all residues at positions 32 to 37 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (38)..(38)
<223> thymidine at position 38 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 310
cgugcagugc cuucggccgt gcggtgccuc cgucacgt 38

<210> 311
<211> 38
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(3)
<223> all residues at positions 1 to 3 are 2'-O-Methyl

<220>
<221> modified_base
<222> (7)..(17)
<223> all residues at positions 7 to 17 are 2'-O-Methyl

<220>
<221> modified_base

<222> (19)..(19)
<223> the residue at position 19 is 2'-O-Methyl

<220>
<221> misc_feature
<222> (19)..(20)
<223> guanosine at position 19 is modified by a phosphorothioate and attached to the thymidine at position 20

<220>
<221> modified_base
<222> (21)..(21)
<223> the residue at position 21 is 2'-O-Methyl

<220>
<221> modified_base
<222> (26)..(27)
<223> all residues at positions 26 to 27 are 2'-O-Methyl

<220>
<221> modified_base
<222> (29)..(29)
<223> the residue at position 29 is 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(37)
<223> all residues at positions 32 to 37 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (38)..(38)
<223> thymidine at position 38 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 311
cgugcagugc cuuuggccgt gcggtgccuc cgucacgt 38

<210> 312
<211> 35
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(3)
<223> all residues at positions 1 to 3 are 2'-O-Methyl

<220>
<221> modified_base
<222> (7)..(11)

<223> all residues at positions 7 to 11 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (11)..(12)
<223> cytidine at position 11 is modified by a PEG and attached to the
guanosine at position 12

<220>
<221> modified_base
<222> (12)..(14)
<223> all residues at positions 12 to 14 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (16)..(17)
<223> guanosine at position 16 is modified by a phosphorothioate and
attached to the thymidine at position 17

<220>
<221> modified_base
<222> (16)..(16)
<223> the residue at 16 is 2'-O-Methyl

<220>
<221> modified_base
<222> (18)..(18)
<223> the residue at 16 is 2'-O-Methyl

<220>
<221> modified_base
<222> (23)..(24)
<223> all residues at positions 23 to 24 are 2'-O-Methyl

<220>
<221> modified_base
<222> (26)..(26)
<223> the residue at position 26 is 2'-O-Methyl

<220>
<221> modified_base
<222> (29)..(30)
<223> all residues at positions 29 to 30 are 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(34)
<223> all residues at positions 32 to 34 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> thymidine at position 35 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 312

cgugcagugc cggccgtgcg gtgccuccgu cacgt

35

<210> 313

<211> 35

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(3)

<223> all residues at positions 1 to 3 are 2'-O-Methyl

<220>

<221> modified_base

<222> (7)..(11)

<223> all residues at positions 7 to 11 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (11)..(12)

<223> cytidine at position 11 is modified by a PEG and attached to the guanosine at position 12

<220>

<221> modified_base

<222> (12)..(14)

<223> all residues at positions 12 to 14 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (16)..(17)

<223> guanosine at position 16 is modified by a phosphorothioate and attached to the thymidine at position 17

<220>

<221> modified_base

<222> (16)..(16)

<223> the residue at position 16 is 2'-O-Methyl

<220>

<221> modified_base

<222> (18)..(18)

<223> the residue at position 18 is 2'-O-Methyl

<220>

<221> modified_base

<222> (23)..(24)

<223> all residues at positions 23 to 24 are 2'-O-Methyl

<220>

<221> modified_base

<222> (26)..(26)
<223> the residue at position 26 is 2'-O-Methyl

<220>
<221> modified_base
<222> (29)..(34)
<223> all residues at positions 29 to 34 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> thymidine at position 35 is 3' inverted deoxythymidine (3' to 3' linked)

<400> 313
cgugcagugc cggccgtgcg gtgccuccgu cacgt 35

<210> 314
<211> 35
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (6)..(7)
<223> guanosine at position 6 is modified by a phosphorothioate and attached to the thymidine at position 7

<220>
<221> modified_base
<222> (6)..(6)
<223> the residue at position 6 is 2'-O-Methyl

<220>
<221> modified_base
<222> (13)..(14)
<223> all residues at positions 13 to 14 are 2'-O-Methyl

<220>
<221> modified_base
<222> (16)..(16)
<223> the residue at position 16 is 2'-O-Methyl

<220>
<221> modified_base
<222> (19)..(23)

<223> all residues at positions 19 to 23 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (23)..(24)
<223> cytidine at position 23 is modified by a PEG and attached to the
guanosine at position 24

<220>
<221> modified_base
<222> (24)..(25)
<223> all residues at positions 24 to 25 are 2'-O-Methyl

<220>
<221> modified_base
<222> (29)..(34)
<223> all residues at positions 29 to 34 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> thymidine at position 35 is a 3' inverted deoxythymidine (3' to
3' linked)

<400> 314
cggccgtgcg gtgccuccgu cacgugcagu gccgt 35

<210> 315
<211> 38
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (6)..(7)
<223> guanosine at position 6 is modified by a phosphorothioate and
attached to the thymidine at position 7

<220>
<221> modified_base
<222> (6)..(6)
<223> the residue at position 6 is 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(8)

<223> the residue at position 8 is 2'-O-Methyl

<220>
<221> modified_base
<222> (13)..(14)
<223> all residues at positions 13-14 are 2'-O-Methyl

<220>
<221> modified_base
<222> (16)..(16)
<223> the residue at position 16 is 2'-O-Methyl

<220>
<221> modified_base
<222> (19)..(28)
<223> all residues at positions 19 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (32)..(37)
<223> all residues at positions 32 to 37 are 2'-O-Methyl

<220>
<221> modified_base
<222> (38)..(38)
<223> the residue at position 38 is 2'-O-Methyl

<400> 315
cggccgtgcg gtgccuccgu cacuuugugc agugccgt 38

<210> 316
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(5)
<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (7)..(8)
<223> guanosine at position 7 is modified by a phosphorothioate and attached to the thymidine at position 8

<220>
<221> modified_base
<222> (7)..(7)
<223> the residue at the 7 position is 2'-O-Methyl

<220>
<221> modified_base
<222> (9)..(9)
<223> the residue at the 9 position is 2'-O-Methyl

<220>
<221> modified_base
<222> (14)..(15)
<223> all residues at positions 14 to 15 are 2'-O-Methyl

<220>
<221> modified_base
<222> (17)..(17)
<223> the residue at position 17 is 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(29)
<223> all residues at positions 20 to 29 are 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(39)
<223> all residues at positions 33 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 316
ccggccgtgc ggtgccuccg ucacuuugug cagugccggt 40

<210> 317
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(5)
<223> all residues at positions 1 to 5 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (7)..(8)
<223> guanosine at position 7 is modified by a phosphorothiolate and attached to the thymidine at position 8

<220>

<221> modified_base
<222> (9)..(9)
<223> the residue at position 9 is 2'-O-Methyl

<220>
<221> modified_base
<222> (14)..(15)
<223> all residues at positions 14 to 15 are 2'-O-Methyl

<220>
<221> modified_base
<222> (17)..(17)
<223> the residue at position 17 is 2'-O-Methyl

<220>
<221> modified_base
<222> (20)..(21)
<223> all residues at positions 20 to 21 are 2'-O-Methyl

<220>
<221> modified_base
<222> (23)..(31)
<223> all residues at positions 23 to 31 are 2'-O-Methyl

<220>
<221> modified_base
<222> (35)..(41)
<223> all residues at positions 35 to 41 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 317
ccggccgtgc ggtgccuccg ucacguuccg ugcagugccg gt 42

<210> 318
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature
<222> (21)..(22)
<223> guanosine at position 21 is modified by a phosphorothioate and attached to the thymidine at position 22

<220>
<221> misc_feature

<222> (42)..(42)
<223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 318
ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 319
<211> 40
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (20)..(21)
<223> guanosine at position 20 is modified by a phosphorothioate and attached to thymidine at position 21

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)

<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>

<221> modified_base

<222> (36)..(39)

<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (40)..(40)

<223> thymidine at position 40 is 3' inverted deoxythymidine (3' to 3' linked)

<400> 319

gcgugcagug ccuucggccg tgcggtgccu ccgucacgct

40

<210> 320

<211> 40

<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: chemically synthesized

<220>

<221> modified_base

<222> (1)..(4)

<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>

<221> modified_base

<222> (1)..(1)

<223> the residue at position 1 is modified with a PEG

<220>

<221> modified_base

<222> (8)..(18)

<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>

<221> misc_feature

<222> (20)..(21)

<223> guanosine at position 20 is modified by a phosphorothioate and attached to the thymidine at position 21

<220>

<221> modified_base

<222> (20)..(20)

<223> the residue at position 20 is 2'-O-Methyl

<220>

<221> modified_base

<222> (22)..(22)

<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> all residues at positions 27 to 28 are 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 320
gcgugcagug ccuucggccg tgcggtgccu ccgucacgct 40

<210> 321
<211> 41
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> modified_base
<222> (1)..(4)
<223> all residues at positions 1 to 4 are 2'-O-Methyl

<220>
<221> modified_base
<222> (1)..(1)
<223> the residue at position 1 is modified with a PEG

<220>
<221> modified_base
<222> (8)..(18)
<223> all residues at positions 8 to 18 are 2'-O-Methyl

<220>

<221> misc_feature
<222> (20)..(21)
<223> guanosine at position 20 is modified by phosphothioate and attached to the thymidine at position 21

<220>
<221> modified_base
<222> (20)..(20)
<223> the residue at position 20 is 2'-O-Methyl

<220>
<221> modified_base
<222> (22)..(22)
<223> the residue at position 22 is 2'-O-Methyl

<220>
<221> modified_base
<222> (27)..(28)
<223> the residue at position 27 to 28 is 2'-O-Methyl

<220>
<221> modified_base
<222> (30)..(30)
<223> the residue at position 30 is 2'-O-Methyl

<220>
<221> modified_base
<222> (33)..(34)
<223> all residues at positions 33 to 34 are 2'-O-Methyl

<220>
<221> modified_base
<222> (36)..(39)
<223> all residues at positions 36 to 39 are 2'-O-Methyl

<220>
<221> misc_feature
<222> (40)..(40)
<223> thymidine at position 40 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 321
gcgugcagug ccuucggccg stgcggtgcc uccgucacgc t 41

<210> 322
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<220>
<221> misc_feature

<222> (42)..(42)
 <223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 322
 ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 323
 <211> 42
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<220>
 <221> modified_base
 <222> '(1)..(1)'
 <223> the residue at position 1 is modified by a PEG

<220>
 <221> misc_feature
 <222> (42)..(42)
 <223> thymidine at position 42 is a 3' inverted deoxythymidine (3' to 3' linked)

<400> 323
 ggcgtgcagt gccttcggcc gtgcggtgcc tccgtcacgc ct 42

<210> 324
 <211> 0
 <212> DNA
 <213> Artificial

<220>
 <223> chemically synthesized

<400> 324
 000

<210> 325
 <211> 80
 <212> DNA
 <213> Artificial

<220>
 <223> Description of Artificial Sequence: chemically synthesized

<400> 325
 ctacctacga tctgactagc ttggtagyga yhyhgwggag mtgcggytg gyygacrtca 60
 gcttactctc atgtagttcc 80

<210> 326
<211> 80
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 326
ctacctacga tctgactagc ctcagattga ctccggcyga cttgttttaa tcttctgagt 60
gcttactctc atgtagttcc 80

<210> 327
<211> 78
<212> DNA
<213> Artificial

<220>
<223> Description of Artificial Sequence: chemically synthesized

<400> 327
ctacctacga tctgactagc cyyacytaty systwcykyr krataygtcg artactmtgc 60
ttactctcat gtagttcc 78

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